Data Evaluation Record on the dissipation and ecological effects of clothianidin (TI-435) in simulated pond (mesocosm) systems.

PMRA Submission Number {.....} EPA MRID Number 47483004

Data Requirement: PMRA Data Code:
EPA DP Barcode: 357014
OECD Data Point:
EPA Guideline: NG

Test material:

Common name:	Clothianidin.
Chemical name:	(E)-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine.
IUPAC name:	(E)-N-(2-Chloro-1,3-thiazol-5-yl)methyl]-N-[oxido(oxo)hydrazono]methanediamine. Chloro-1,3-thiazol-5-yl)methyl]-N-{(E)-(methylamino)[oxido(oxo)-hydrazono]methyl}amine.
CAS name:	[C(E)]-N-[(2-Chloro-5-thiazolyl)methyl]-N′-methyl-N″-nitroguanidine.
CAS No:	210880-92-5 (formerly 205510-53-8).
Synonyms:	TI-435, C-1015, C-908, TI435, K-1142, TI-435 50 WDG, TI-435 50WDG.
SMILES string:	CNC(=N[N+](=O)O)NCc1cnc(Cl)s1 (Online SMILES Translator and Structure File Generator at http://cactus.nci.nih.gov/services/translate/). [O-][N+](=O)N=C(NCc1cnc(s1)Cl)NC.

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Company Code:
Active Code:
Use Site Category:
EPA PC Code: 044309

CITATION: Memmert, U. 2001. Fate and ecological effects of TI-435 50 WG in a outdoor freshwater mesocosm study. Unpublished study performed by RCC Ltd., Itingen, Switzerland, and Aachen University of Technology, Department of Biology V (Ecology, Ecotoxicology, Ecochemistry), Aachen, Germany; sponsored by Takeda Chemical Industries, Ltd., Tokyo, Japan; and submitted by Bayer CropScience (pp. 1-2, 17). RCC Study No.: 753851 (p. 1).

Experiment start date May 3, 2000, and completion date January 20, 2001 (p. 18). Final report issued March 14, 2001 (p. 1).

Results for clothianidin residues in the pond water and sediment presented in the study report were calculated based on the amount of TI-435 50WG formulated product added to the test systems and not on the actual amount of clothianidin active ingredient added (Attachment I, p. A28; Table 4, pp. A36-A38). Consequently, for all clothianidin residue results presented in this DER, the primary reviewer recalculated the provided data to reflect residue levels of the active ingredient (DER Attachment 2).

EXECUTIVE SUMMARY

The dissipation and toxicity effects of (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine (clothianidin, TI-435) were studied for 98 days in outdoor, simulated pond systems comprised of water (pH 7.1-7.4, total organic carbon 12.6-15.7 mg/L) and sediment (pH 7.1-7.2, organic carbon 1.5-2.0%) obtained from sources located near Aachen and Roetgen, Germany. Water was obtained from previously established mesocosm study control ponds, tap water from a local reservoir and a natural pond. Sediment was primarily obtained from a natural pond supplemented with sediment from previously established mesocosm control ponds. Clothianidin was applied as an end-use, formulated, water dispersible granule (TI-435 50WG) solution sprayed directly onto the water surface to yield nominal initial concentrations of 0.10, 0.31, 1.0, 3.1 and 10 µg a.i./L. This study was conducted in accordance with SETAC Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (1991), and OECD Draft Proposal for a Guidance Document "Freshwater Lentic Field Tests" (1996), and in compliance with Swiss GLP Ordinance RS 813.016.5 (2000), with the following exclusions:

- work conducted during establishment of the test systems including filling the ponds with sediment and water, stocking with flora and fauna, circulation of water, measurements of water and sediment parameters, sampling, determination and counting of the organisms;
- recording of meteorological data;
- analysis of the local tap water by the water supplier; and
- analytical pre-experiments (method validations).

The test apparatus consisted of thirteen cylindrical glass-fiber reinforced polyester tanks (volume *ca*. 3.4-4.2 m^3) set into the ground to a depth of *ca*. 1 m. The bottom of each tank was covered with sand, which was overlaid with sediment (*ca*. 10-cm depth) and water (*ca*. 1.1-m depth, *ca*. 3,500-4,200 L). The pond systems were allowed to acclimate for 92 days prior to treatment, with circulation of water between ponds during the acclimatization period. At 1-3 weeks prior to treatment, emergence (insect) traps were positioned on the water surface, macroinvertebrate artificial substrate sampler (MASS) units were positioned on the sediment surface, inter-pond water circulation was stopped, and the ponds were amended with the green algal species *Scenedesmus subspicatus*. Duplicate pond systems were treated at each application rate, with the remaining three ponds maintained as untreated controls. For clothianidin analyses, water samples (all treatment rates) were taken at 2, 4, 7, 14, 28, 42, 56, 70, 84 and 98 days posttreatment, with sediment (3.1 and 10 µg a.i./L rates) taken at the same intervals from 7 to 98 days. Sampled water and sediment were stored frozen (≤-20ºC) prior to processing and analysis. For biota, water (phytoplankton, zooplankton), sediment (benthic macroinvertebrates), MASS

units (benthic macroinvertebrates) and emergence traps (insects) were sampled at regular intervals up to 98 days posttreatment. Duckweed (*Lemna sp.*) and filamentous periphyton were periodically removed as maintenance procedures.

Water samples from ponds treated at 10 and 3.1 µg a.i./L were analyzed directly by HPLC/UV. All other water samples were concentrated via C-18 solid-phase extraction (SPE) cartridge followed by rotary evaporation, then residues were reconstituted in water and analyzed by HPLC/UV or LC/MS/MS. Sediment samples were extracted once with water:acetonitrile:acetic acid (800:200:0.8, v:v) and the resulting extract filtered (filter not specified) once, then again (0.45-µm) prior to LC/MS/MS analysis. For phytoplankton, water samples were preserved with Lugol's solution for taxonomy and counts, with additional water samples analyzed for chlorophyll-a and phaeophytin via UV. For zooplankton, water samples were sieved (55-µm) and the retained organisms fixed in 95% ethanol followed by preservation in 70% ethanol:40 g/L saccharose:40 ml/L glycerine. Benthic organisms were recovered from sediment and the MASS units by sieving (*ca*. 1-mm and 1.5-mm mesh, respectively), with the retained organisms preserved in 70% ethanol:40 g/L saccharose:40 ml/L glycerine. Emergence traps contained the same preservation solution used for the benthic organisms which preserved the insects upon trapping. Duckweed and periphyton were analyzed for total biomass.

Test conditions. The pond systems were maintained outdoors under natural conditions. Pond water levels were adjusted just prior to treatment with no water added throughout the 98-day study. In the treated pond systems, mean (n = 10) water temperature increased from 14.3 ± 0.1ºC at day 0 posttreatment to 20.1 ± 0.4ºC at study termination (98 days). Additional parameters in the water layer of the treated pond systems over the study duration were as follows: pH 7.8 ± 0.7 (n = 120), dissolved oxygen 7.2 ± 3.7 mg/L (n = 110), conductivity 252 ± 16 µS/cm (n = 120), total organic carbon 17.2 ± 4.6 mg/L (n = 50), hardness 0.97 ± 0.06 mmol/L (n = 50), total nitrogen 3.65 ± 1.33 mg N/L (n = 50), total phosphorus 0.36 ± 0.16 mg P/L (n = 50) and depth 110.8 ± 4.3 cm (n = 130). Diurnal measurements at 7, 56 and 98 days, found gradual increases in dissolved oxygen concentration, pH and temperature throughout the day, while conductivity remained relatively steady-state. Conditions in the treated pond systems were comparable to those measured in the untreated control pond systems. Meteorological conditions were monitored by a nearby weather station; however, the data were not provided.

Residue analyses. Water. Increasing clothianidin treatment rate resulted in increasing persistence of the test material in the water layer. At the 0.10 µg a.i./L rate, clothianidin residues decreased from a mean 0.0583 µg a.i./L (59% of nominal applied) at 2 days posttreatment (first interval analyzed) to 0.0551 µg a.i./L (56%) at 4 days, 0.0481 µg a.i./L (49%) at 7 days, 0.0266 µg a.i./L (27%) at 28 days, 0.0187 µg a.i./L (19%) at 42 days and were <LOD (<0.015 µg a.i./L) at 56-98 days. At the 0.31 µg a.i./L rate, clothianidin residues decreased from a mean 0.245 µg a.i./L (79% of applied) at 2 days to 0.207 µg a.i./L (66%) at 4 days, 0.141 µg a.i./L (45%) at 7 days, 0.0679 µg a.i./L (22%) at 28 days, 0.0331 µg a.i./L (11%) at 56 days and were 0.0183 µg a.i./L (6%) at 98 days. At the 1.0 µg a.i./L rate, clothianidin residues decreased from a mean 0.92 µg a.i./L (94% of applied) at 2 days to 0.67 µg a.i./L (68%) at 7 days, 0.455 µg a.i./L (46%) at 14 days, 0.192 µg a.i./L (19%) at 42 days, 0.090 µg a.i./L (9%) at 70 days and were 0.047 µg

a.i./L (5%) at 98 days. At the 3.1 µg a.i./L rate, clothianidin residues decreased from a mean 2.92 µg a.i./L (94% of applied) at 2 days to 2.20 µg a.i./L (71%) at 14 days, 1.32 µg a.i./L (42%) at 28 days, 0.67 µg a.i./L (22%) at 56 days and were 0.295 µg a.i./L (9%) at 98 days. At the 10 µg a.i./L rate, clothianidin residues decreased from a mean 9.88 µg a.i./L (100% of applied) at 2 days to 5.29 µg a.i./L (54%) at 14 days, 4.67 µg a.i./L (47%) at 28 days, 2.18 µg a.i./L (22%) at 56 days, 1.02 µg a.i./L (10%) at 84 days and were 0.76 µg a.i./L (8%) at 98 days.

Half-life/DT50 in water: Observed DT50 values for clothianidin were *ca*. 6-7 days at the 0.10-0.31 µg a.i./L rates, *ca*. 13 days at the 1.0 µg a.i./L rate and *ca*. 22-24 at the 3.1-10 µg a.i./L rates. Calculated linear (Excel 2007) half-lives were 22-24 days (r^2 = 0.8705-0.9743) at the 0.10-1.0 µg a.i./L rates and 26-29 days (r^2 = 0.9840-0.9860) at the 3.1-10 µg a.i./L rates, with respective nonlinear (SigmaPlot v 9, exponential decay/single compartment, 2 parameter) half-lives of 15-18 days (r^2 = 0.9629-0.9786) and 24-27 days (r^2 = 0.9916-0.9967).

Sediment. Only sediment from ponds treated at 3.1 and 10 µg a.i./L were analyzed for clothianidin. Increased clothianidin treatment rate resulted in higher levels and persistence of the test material in the sediment. At the 3.1 µg a.i./L rate, clothianidin residues were initially detected at a mean 5.0 µg a.i./kg at 28 days decreasing to 2.6 µg a.i./kg at 56 days, with sediment collected at 70-98 days not analyzed. At the 10 µg a.i./L rate, clothianidin residues were detected at a mean 12.6 µg a.i./kg at 7 days (first interval analyzed), were a maximum 15.2 µg a.i./kg at 28 days, then decreased to 6.9 µg a.i./kg at 84 days and were 4.2 µg a.i./kg at 98 days.

Half-life/DT50 in sediment: Observed DT50 values for clothianidin were *ca*. 56 and 79 days at the 3.1 and 10 µg a.i./L rates, respectively. Calculated linear half-lives (r^2 = 0.6089-0.6470) were 27 and 42 days at the 3.1 and 10 µg a.i./L rates, respectively, with respective nonlinear half-lives (r^2 = 0.9244-0.9635) of 33 and 45 days.

Ecological effects. Phytoplankton (30 taxa). There were no consistent concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L (no observed effect concentration [NOEC]), on community parameters (taxa abundance, diversity, evenness, similarity, principle response curve analyses) or population densities. Additionally, levels of chlorophyll-a and phaeophytin indicated clothianidin, up to 10 µg a.i./L (NOEC), had no effect on total phytoplankton biomass.

Zooplankton (22 taxa). There were no consistent concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L (NOEC), on community parameters or population densities for individual and total taxa. However, concentration-effect analyses of population densities for total cladocerans, total copepods and total rotifers found a statistically significant ($p \leq 0.05$) negative trend for total rotifers during the 70-98 day posttreatment interval, yielding EC20 and EC50 values of 9.5 and 10.5 µg a.i./L, respectively.

Benthic macroinvertebrates (7 taxa). For benthic organisms (7 taxa) recovered from MASS units positioned on the sediment surface, there were no consistent concentration-dependent toxic

effects attributable to clothianidin, up to 10 µg a.i./L (NOEC), on community parameters or population densities.

For sediment dwelling benthic organisms (5 taxa), there were no consistent concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L (NOEC), on taxa abundance or similarity. However, diversity and evenness showed toxic effects at both the 3.1 and 10 µg a.i./L treatment rates, with statistically significant ($p \leq 0.05$) EC20 and EC50 values (7-14 days posttreatment) of 2.0 and 9.2 µg a.i./L, respectively, for diversity and 3.3 and 15.3 µg a.i./L, respectively, for evenness. The decreases in diversity and evenness were believed due toxicity of clothianidin on chironomid larvae with a NOEC value of 1.0 µg a.i/L at 7 days; however, population density recovered to control levels by 28 days posttreatment. There were no consistent concentration-dependent toxic effects (NOEC 10 µg a.i./L) on population densities of the other recovered benthic macroinvertebrate taxa (tubificid worms, water snails and leeches).

Emergent insects (16 taxa). The insect populations were dominated by chironomid taxa which comprised 10 of the 16 total taxa. There were no consistent concentration-dependent toxic effects on community parameters attributable to clothianidin up to 1 µg a.i./L (NOEC); however, at both the 3.1 and 10 µg a.i/L treatment rates all parameters showed transient toxic effects. Statistically significant ($p \leq 0.05$) EC20 values (7-21 days posttreatment) for taxa abundance, diversity, evenness and similarity (Steinhaus') were 1.2, 1.4, 3.1 and 2.2 µg a.i./L, respectively, with respective EC50 values of 3.1, 2.9, 3.8 and 3.0 µg a.i./L. Similarly, there were no consistent concentration-dependent toxic effects attributable to clothianidin up to 1 µg a.i./L (NOEC) on total emergent insect population densities, but population densities did show toxic effects at both the 3.1 and 10 µg a.i/L rates with EC20 values of 1.0 µg a.i./L (not statistically significant) at 7-21 days posttreatment and 1.4 µg a.i./L ($p \leq 0.05$) at 28-63 days, with respective EC50 values of 1.7 and 7.7 µg a.i./L. Population densities of all affected insects as well as all community parameters recovered to control levels by 77 days posttreatment.

Macrophytes and periphyton. There were no observable concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L, on the total biomass of duckweed (*Lemma sp.*) and filamentous periphyton.

Supplemental experiments. Method validation for clothianidin in water. For pond water samples analyzed via HPLC/UV, overall recovery from pond water fortified with clothianidin at 0.105-10.48 µg a.i./L averaged (n = 8) 95 ± 6% (range 84-103%) of the applied. For pond water samples analyzed via LC/MS/MS, overall recovery from pond water fortified with clothianidin at 5.24 µg a.i./L averaged (n = 2) 88 ± 0% (range 88-89%) of the applied; however, recoveries from pond water fortified at 0.0262-0.262 µg a.i./L only averaged (n = 14) 64 ± 5% (range 56-73%) of the applied. Consequently, results from all pond water samples analyzed via LC/MS/MS for this study were corrected by the mean recovery value of 64%.

Method validation for clothianidin in sediment. Overall recovery from sediment fortified with clothianidin at 7.71-84.9 µg a.i./kg averaged (n = 16) 104 ± 9% (range 91-123%) of the applied.

Study Acceptability: This study is classified as [to be filled in by the EFED reviewer]. The following significant deviations from good scientific practices or the objectives of OPPTS guidelines were noted:

- application rates were not confirmed for the ponds treated at the 0.10 and 0.31 µg a.i./L test rates,
- the volumes of the test mesocosms were insufficient,
- the sediment depth was insufficient,
- finfish species were not included,
- the number of pond replicates for test material treatment levels was insufficient, and
- clothianidin residue analyses were incomplete.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with SETAC Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (1991), and OECD Draft Proposal for a Guidance Document "Freshwater Lentic Field Tests" (1996, pp. 19-20). The following significant deviation from good scientific practices and/or the objectives of OPPTS guidelines were noted:

Application rates were not confirmed for the ponds treated at the 0.10 and 0.31 µg a.i./L test rates. Following spray application, water samples were not taken until 2 days posttreatment, at which time clothianidin comprised only 59 ± 5% and 79 ± 2% of the applied in the 0.10 and 0.31 µg a.i./L treated ponds, respectively. Analysis of the formulated test solutions, taken just prior to application, indicated no degradation of clothianidin; however, no verification procedures were performed during spray application of the formulated solution to the test ponds.

The mesocosm volumes were only *ca*. 3.4-4.2 m^3 (based on water surface areas of *ca*. 3.1 m^2 for ten of the ponds and *ca*. 3.8 m^2 for three of the ponds, and a water depth of *ca*. 1.1 m for all ponds). Guidelines specify a minimum mesocosm volume of 300 m^3.

The sediment depth in the mesocosms was *ca*. 10 cm. Guidelines specify sediment depth should be a minimum of 15 cm.

Finfish were not included because the mesocosm were too small to accomodate finfish species. Guidelines specify mesocosm dimensions must be sufficient to accommodate a viable finfish population.

Only two replicates per treatment level were conducted for the clothianidin treated mesocosms, with three replicates utilized for the untreated control ponds. Guidelines specify a minimum of three replicates per treatment level.

Water and sediments were only analyzed for parent clothianidin, and biota were not subjected to any residue analyses. Guidelines specify that residues of the test material and major degradates/metabolites are to be analyzed at appropriate intervals in the water, sediments and biota.

COMPLIANCE: This study was conducted in compliance with Swiss GLP Ordinance RS 813.016.5 (2000; pp. 3-4, 7), with the following exclusions:

- Work conducted during establishment of the test systems including filling the ponds with sediment and water, stocking with flora and fauna, circulation of water, measurements of water and sediment parameters, sampling, determination and counting of the organisms.
- Recording of meteorological data.
- Analysis of the local tap water by the water supplier.
- Analytical pre-experiments (method validations).

The study author reported that there were no circumstances that affected the quality or integrity of the data (pp. 3-4). Signed and dated Data Confidentiality, GLP, Quality Assurance statements, and Signatures page were provided (pp. 2-4, 6-8).

A. MATERIALS:

1. Test Material		Clothianidin (TI-435 50WG, p. 25).
Chemical Structure:		See DER Attachment 1.
Description:		Solid, water dispersible (WG or WDG) formulation (p. 25).
Purity:	Analytical purity:	49.3% (pp. 20, 25; Attachment I, p. A6).
	Lot/Batch No.:	IW016-046 (p. 25).
	Radiochemical purity:	Not applicable.

Specific activity: Not applicable.

Location of the radiolabel: Not applicable.

Storage conditions of test chemical: The test substance was stored at room temperature (*ca*. 20ºC) in darkness (p. 26).

Physico-chemical properties of clothianidin:

Parameter		Value	Comment
Molecular weight		249.68 g/mol.	
Molecular formula		$C_6H_8ClN_5O_2S$.	
Water solubility		0.270 g/L.	At 20ºC.
Vapor Pressure/Volatility		5.1 x 10^{-11} Pa.	At 25ºC.
UV Absorption		Not reported.	
Dissociation constant (pKa)		Not reported.	
Partition coefficient (octanol/water), log K_{ow}		1.05.	At 24ºC.
Stability of compound at room temperature		Not reported.	
Stability under the following conditions:			
Hydrolysis:	At pH 4 and 7:	Stable; no hydrolysis at 50ºC after 5 days.	
	At pH 9:	Half-life of 1,393 days at 20ºC.	
Photolysis:	pH 7 buffer solution:	Half-life of <1 day.	
	Natural water and sunlight:	Half-life of 1-5 days.	
Water-sediment systems:	In total system:	DT50 values of 34, 60 days.	

Data obtained from pp. 25-26 of the study report.

2. Water-sediment collection, storage and properties

Table 1: Description of water-sediment collection and storage.

Description		Details
Geographic location	Water:	Water was obtained from non-polluted control ponds, established in April 1998, for prior mesocosm studies conducted at Aachen University of Technology, Department of Biology V (Ecology, Ecotoxicology, Ecochemistry), Aachen, Germany; pp. 17, 29, 36). Local tap water was obtained from the Dreilägerbach-Talsperre water reservoir located near Roetgen, Germany, with ground water mixed into the tap water by the supplier (p. 17; Figure 4, p. 35; p. 36). Additionally, water was obtained from the non-polluted natural pond Alsdorfer Weiher[1], on March 15, 2000, to provide an additional source of phytoplankton and zooplankton (Figure 3, p. 34; p. 38).
	Sediment:	The majority of the sediment was collected from the natural, non-polluted pond Hasselholzer Weiher, Aachen, Germany (pp. 17, 29; Figure 2, p. 33). Additional sediment was obtained from the previously established (1998) control mesocosm ponds described above; sediment for these ponds was also initially obtained from the Hasselholzer Weiher (p. 29).
Pesticide use history at the collection site[2]		Sites were described as “non-polluted” (pp. 29, 36).

Table 1: Description of water-sediment collection and storage.

Description		**Details**
Collection date	Water:	From previously established (1998) control mesocosm ponds: January 19, 2000 (pp. 29, 36).
	Sediment:	From Hasselholzer Weiher: January 18, 2000 (p. 29). From previously established (1998) control mesocosm ponds: January 19, 2000 (p. 29).
Collection procedures for	Water:	Not reported.
	Sediment:	Macrophytes were removed using an excavator, then the sediment was excavated, overlying water was removed, and the sediment was transported to the test facility in a container; no additional details were provided (p. 29).
Sampling depth for	Water:	From previously established (1998) control mesocosm ponds: not reported. From Alsdorfer Weiher: surface water (p. 38).
	Sediment:	*ca*. 0-0.6 m depth (p. 29).
Storage conditions	Water:	None; water from previously established (1998) control mesocosm ponds was used the day of collection (p. 36).
	Sediment:	Not reported.
Storage length	Water:	None; water from previously established (1998) control mesocosm ponds was used the day of collection (p. 36).
	Sediment:	From Hasselholzer Weiher: overnight (p. 29). From previously established (1998) control mesocosm ponds: none (p. 29).
Preparation	Water:	None reported.
	Sediment:	None reported.

1 Weiher translates as "pond".

2 Residue analyses of sediment from three of the thirteen established simulated pond systems (see **Table 3. Properties of the sediment** below) found the pesticides lindane, heptachlor, malathion, DDT and dieldrin and PCBs were all below limits of detection (Table 1, p. 30).

Data obtained from pp. 17, 29; Table 1, p. 30; Figures 2-4, p. 33-35; pp. 36, 38 of the study report.

Table 2: Properties of the water.[1, 2]

Property	**Treated Pond Systems (n = 10)**		**Control Pond Systems (n = 3)**	
Temperature (ºC)	Initial:	Final:	Initial:	Final:
	14.3 ± 0.1	20.1 ± 0.4	14.4 ± 0.1	19.9 ± 0.4
pH	Initial:	Final:	Initial:	Final:
	7.3 ± 0.0	8.4 ± 1.1	7.2 ± 0.1	9.0 ± 1.2
Redox potential (mV)	Initial:	Final:	Initial:	Final:
	Not reported.			
Oxygen concentration (mg/L)	Initial:	Final:	Initial:	Final:
	2.3 ± 0.8	9.5 ± 5.6	2.3 ± 0.4	11.8 ± 3.5
Dissolved organic carbon (%)	Initial:	Final:	Initial:	Final:
	Not reported.			
Total organic carbon (mg/L)	Initial:	Final:	Initial:	Final:
	14.0 ± 0.9	14.5 ± 2.1	13.1 ± 0.4	14.6 ± 2.3

Table 2: Properties of the water.[1, 2]

Property	Treated Pond Systems (n = 10)		Control Pond Systems (n = 3)	
Hardness (mmol/L)	Initial:	Final:	Initial:	Final:
	0.95 ± 0.03	0.94 ± 0.06	0.95 ± 0.00	0.89 ± 0.13
Electrical conductivity (µS/cm)	Initial:	Final:	Initial:	Final:
	262 ± 4	219 ± 9	266 ± 1	208 ± 17
Ammonium (NH_4^+, mg N/L)	Initial:	Final:	Initial:	Final:
	3.26 ± 0.13	0.03 ± 0.02	3.49 ± 0.07	0.15 ± 0.15
Nitrite (NO_2^-, mg N/L)	Initial:	Final:	Initial:	Final:
	0.12 ± 0.02	0.01 ± 0.01	0.09 ± 0.02	0.06 ± 0.04
Nitrate (NO_3^-, mg N/L)	Initial:	Final:	Initial:	Final:
	0.52 ± 0.08	0.11 ± 0.06	0.50 ± 0.05	0.31 ± 0.20
Total nitrogen (mg N/L)	Initial:	Final:	Initial:	Final:
	5.39 ± 0.11	2.07 ± 0.24	5.62 ± 0.26	2.56 ± 0.23
Dissolved ortho-phosphate (H_3PO_4, mg P/L)	Initial:	Final:	Initial:	Final:
	0.15 ± 0.04	0.12 ± 0.06	0.18 ± 0.04	0.13 ± 0.07
Total phosphorous (mg P/L)	Initial:	Final:	Initial:	Final:
	0.31 ± 0.08	0.38 ± 0.15	0.32 ± 0.10	0.39 ± 0.11
Microbial biomass/population (units)	Initial:	Final:	Initial:	Final:
	Not reported.			

1 Means and standard deviations calculated by the primary reviewer using data obtained from Attachment II, Tables 94-105, pp. B58-B61 of the study report (Reviewer's Comment No. 1, DER Attachment 2).

2 Initial and Final measured in treated pond systems at 0 and 98 days posttreatment, respectively.

Table 3: Properties of the sediment.[1]

Property	Pond 1		Pond 10		Pond 13	
Soil texture (Table 1, p. 30; p. 31)	Silt loam		Silt loam		Loam	
% Sand (50-2000 µm):	26.2		21.6		46.5	
% Silt (2-50 µm):	54.2		56.3		37.1	
% Clay (<2 µm):	19.6		22.1		16.4	
pH (in $CaCl_2$ suspension)	7.1		7.1		7.2	
Organic carbon (g C/kg dry wt.)	20.0		19.7		14.9	
Organic carbon (%)	2.0		2.0		1.5	
Organic matter (%)[2]	3.4		3.4		2.6	
CEC (mVal/kg dry wt.)	202		185		196	
Redox potential (mV)	Initial:	Final:	Initial:	Final:	Initial:	Final:
	Not reported.					
Bulk density (disturbed, g/cm^3)	Not reported.					
Total heavy metals (mg/kg dry wt.)						
Cadmium:	1.8		1.7		1.9	
Copper:	25		24		26	

Table 3: Properties of the sediment.[1]

Property	Pond 1	Pond 10	Pond 13
Lead:	90	85	94
Zinc:	278	285	284
Dry weight (kg fresh wt./kg dry wt.)	2.0	2.0	2.1
Microbial biomass/population (units)	Initial:	Final:	
	Not reported.		

1 Thirteen outdoor simulated pond systems were established as described (see **Table 5. Study design** below). Sediment samples (*ca*. 1 L per pond) were taken from three of the thirteen pond systems for characterization (p. 29). The sediment was sampled on February 28, 2000; 40 days after establishment of the pond systems (January 19, 2000; p. 29), and 65 days prior to treatment (May 3, 2000; p. 39).

2 Percent organic matter determined by primary reviewer using the following formula: organic matter (%) = organic carbon (%) x 1.72.

Data obtained from p. 29; Table 1, p. 30; pp. 31, 39 of the study report.

Table 4: Introduced biota.

Flora/Fauna	Details
Phytoplankton	*Scenedesmus subspicatus* (strain No. 86.81 SAG), a green algal species, was obtained from Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, Göttingen, Germany (p. 38). The algae was cultured in algal growth medium (Kuhl medium) at the site facility (p. 38).
Other details, if any	The sediment dwelling organisms, phytoplankton and zooplankton in the collected sediment and water were indigenous to the local natural pond Hasselholzer Weiher and the previously established (1998) control mesocosm ponds located at the test site (Figure 2, p. 33; pp. 29, 38). As an additional source of phytoplankton and zooplankton, water (*ca*. 3.5 L) from the local natural pond Alsdorfer Weiher was added to each simulated pond system on March 15, 2000 (Figure 3, p. 34; p. 38).

Data obtained from Figures 2-3, pp. 29, 33-34; p. 38 of the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None reported.

2. Experimental conditions:

Table 5: Study design.

Parameters	Details
Duration of the test (posttreatment)	98 days (Table 5, p. 44).
Water:	
Filtered/unfiltered water:	Unfiltered (p. 36).
Type and size of filter used, if any:	Not applicable.

Table 5: Study design.

Parameters		**Details**
Amount of sediment and water per treatment	Water:	Pond systems 1-10: *ca*. 3,500 L (p. 27; Figure 1, p. 28). Pond systems 11-13: *ca*. 4,200 L (p. 27; Figure 1, p. 28). Initial water depth for all pond systems *ca*. 1.1 m (p. 27).
	Sediment:	All pond systems: *ca*. 10 cm in depth (p. 27).
Application rate	Nominal:	0.10, 0.31, 1.0, 3.1 and 10 µg a.i./L (0.20, 0.63, 2.0, 6.3 and 20 µg formulation/L; Attachment I, pp. A36-A38).
	Actual:	0.10-0.12, 0.32, 1.0, 3.2 and 9.9-10 µg a.i./L (0.21-0.25, 0.65, 2.0-2.1, 6.4, 20-21 µg formulation/L).[1]
No. of replications	Control:	Three untreated control pond systems were prepared and maintained in the same manner as the treated pond systems (p. 27; Figure 1, p. 28).
	Treated:	Duplicate pond systems were treated at each application rate (p. 27; Figure 1, p. 28).
Test apparatus (type/material/volume):		All pond systems: Each pond container consisted of a cylindrical glass-fiber reinforced polyester tank set into the ground to a depth of *ca*. 1 m (p. 27). The tank sides extended 0.2-0.3 m above the water surface; sufficient height to prevent overflow during heavy rainfall (p. 27). Pond systems 1-10: Each pond tank (diameter *ca*. 2 m, surface area *ca*. 3.1 m^2) was filled with sand to a depth of *ca*. 0.45-0.55m, which was overlaid with sediment (*ca*. 10 cm depth) and water (*ca*. 3,500 L, p. 27). Pond systems 11-13: Each pond tank (diameter *ca*. 2.2 m, surface area *ca*. 3.8 m^2) had a conical bottom that was completely filled with sand, then the sand layer was overlaid with sediment (*ca*. 10 cm depth) and water (*ca*. 4,200 L, p. 27). Emergence traps: Three weeks prior to treatment (April 12, 2000), a floating emerging insect (chironomids) trap (base 0.25 m^2, Eco TecH) was positioned in the middle of the water surface of each pond (p. 47). The trap was pyramid-shaped and held a 1-L collection bottle containing preservation solution (70% ethanol, 40 g/L saccharose, 40 ml/L glycerine; p. 47; Figure 5, p. 49). The traps were removed during the test solution application (p. 47). Macroinvertebrate Artificial Substrate Samplers (MASS): Substrate samplers were made from plastic cylinders (diameter 5 cm) used for surface area enhancement in sewage treatment plants (p. 47). Six sections (height 5 cm) of plastic cylinder were fastened around a seventh central section of cylinder, then this unit was fastened on top of a second, similarly prepared, unit (p. 47; Figure 5, p. 49). The base of the substrate sampler was covered with gauze (1-mm mesh), and the unit was weighted with two small plastic bottles containing gravel. Two weeks prior to treatment (April 19, 2000), two MASS units were placed on the sediment surface in each pond (p. 47).
Test system preparation/acclimatization		Sediment allocation: For uniform composition, aliquots (*ca*. 15-20 L per aliquot) of the Hasselholzer Weiher[2] sediment were distributed among the pond tanks on January 19, 2000, with each tank receiving a total of twenty-five aliquots (p. 29). The sediment was spread, using a rake, to obtain a layer depth of *ca*. 8 cm; larger stones were manually removed (p. 29). This sediment layer was overlaid with *ca*. 2-cm layer of sediment obtained from previously established (1998) control mesocosm ponds (see **Table 1: Description of water-sediment collection and storage** above), yielding a total sediment layer of *ca*. 10 cm (p. 29). Water addition: The sediment was immediately covered with water (*ca*.

Table 5: Study design.

Parameters		**Details**
		30-cm depth) also obtained from the previously established (1998) control mesocosm ponds (p. 36). The ponds were further filled with local tap water, from January 25 to February 01, 2000, without disturbing the sediment layer (p. 36). System acclimatization: The pond systems were allowed to acclimate for 92 days (13 weeks, February 1 to May 3, 2000) prior to treatment (p. 39). On weekdays, from March 9 to April 26, 2000, water was slowly circulated between all the ponds through wide tubes (5-cm diameter) using an aquarium pump (flow rate *ca*. 5 m^3 per day) for *ca*. 8 hours per day (p. 39). Circulation of the water ceased 7 days prior to treatment (April 26, 2000) and the tubes were removed (p. 39). On day 43 of the acclimatization (March 15, 2000), each pond system was amended with natural pond water (Alsdorfer Weiher, *ca*. 3.5 L) as an additional source of phytoplankton and zooplankton (p. 38). Prior to treatment (April 26, 2000), each pond system was supplemented with a 1-L suspension (cell density *ca*. 1.3 x 10^6 cells/mL) of the green algal species *Scenedesmus subspicatus* (p. 38; see **Table 4: Introduced biota** above). At study initiation, just prior to treatment (May 3, 2000), the water level in all of the pond systems was adjusted to a depth of *ca*. 1.1 m (p. 36).
Finfish species	Weight (g wet wt.)	Not utilized as the ponds were too small to accommodate finfish which would have significantly influenced the biotic community through predation on zooplankton and benthic organisms (p. 38).
	Length (mm)	
	Feeding regimen	
Test material application method	Volume of test solution used/treatment:	900 mL (Table 3, p. 41).
	Application method:	Spraying; a spray boom was held 1-20 cm above the water surface and passed over the surface several times during application (p. 41). Following test solution application, tap water (300 mL) was added to the sprayer tank and applied to the water surface (p. 41). Ponds not being sprayed were covered the plastic foil to avoid spray drift contamination (p. 41).
	Type of spray equipment, if used:	Hand-held spray boom (Gloria 172 RTG) with a conventional hydraulic nozzle (p. 41).
Identification and volume of carrier, if used		Local tap water, 1,000 mL (p. 40).
Name and concentration of co-solvents, adjuvants and/or surfactants, if used		None used.
Meteorological conditions during application	Cloud cover:	Cloudy, drizzle (Table 4, p. 42).
	Air temperature:	11.5ºC.
	Wind speed:	0.1 m/s.
Other details, if any		None.

1 Actual application rates based on purity (49.3%) clothianidin in formulation, clothianidin measured in application solution samples, final application solution volume (900 mL) added to the ponds systems and pond systems volumes of 3,500 L or 4,200 L (Table 3, p. 41; Attachment I, p. A6; Table 3, p. A35).

2 Weiher translates as “pond”.

Data were obtained from p. 27; Figure 1, p. 28; pp. 29, 36, 38-41; Table 3-4, pp. 41-42; Table 5, p. 44; p. 47; Figure 5, p. 49; Attachment I, pp. A35-A38 of the study report.

3. Application verification: Duplicate aliquots (50 mL) of the formulated solutions were taken just prior to application and stored deep-frozen (≤delete space -20ºC) until analysis (pp. 40, 54-55). Application solution samples (100 mL) with nominal concentrations of 0.39-3.84 mg a.i./L (0.77-7.68 mg formulation/L) were thawed and mechanically shaken (mechanism, speed, interval not reported), then analyzed directly by HPLC/UV as described below (Attachment I, p. A10; Table 3, p. A35). Application solution samples at higher concentrations (12.1-46.4 mg a.i./L, 24.18-92.87 mg formulation/L) were diluted with tap water prior to analysis (Attachment I, p. A10; Table 3, p. A35).

4. Environmental conditions: The treated systems were maintained outdoors under natural conditions. Dissolved oxygen, pH, temperature, conductivity, hardness, ammonium, nitrite, nitrate, total nitrogen, dissolved ortho-phosphate, total phosphorus and total organic carbon in the water layer and the water level of the pond systems were measured prior to and following treatment (Attachment II, pp. B58-B64). Meteorological conditions, including air temperature (mean, minimum, maximum), barometer reading, precipitation, relative air humidity, solar radiation, wind speed and primary wind direction, were monitored by a weather station located *ca*. 200 m from the location of the pond systems; however, the recorded meteorological data were not provided, but archived with the study data (p. 53).

5. Supplementary experiments: Method validation for clothianidin in water. Tap water and untreated pond water were fortified with formulated TI-435 50WG as follows: tap water containing clothianidin at 1.75 µg a.i./L, 0.350 mg a.i./L and 46.6 mg a.i./L (3.54 µg/L, 0.709 mg/L and 94.5 mg/L TI-435 50WG, respectively), and pond water containing clothianidin at 0.0262, 0.105, 0.262, 0.350, 5.24 and 10.5 µg a.i./L (0.0532, 0.213, 0.532, 0.709, 10.63 and 21.3 µg TI-435 50WG/L, respectively; Attachment I, p. A9). Fortified water samples were either analyzed directly by HPLC/UV, or extracted and analyzed by HPLC/UV and/or LC/MS/MS as described below (Attachment I, p. A9).

Method validation for clothianidin in sediment. Untreated pond sediment (*ca*. 20 g) were fortified with formulated TI-435 50WG to contain clothianidin at *ca*. 8, 24 and 80 µg a.i./kg wet sediment (*ca*. 16, 48 and 160 µg TI-435 50WG/kg wet sediment, respectively; Attachment I, p. A18; Table 7, p. A41). Fortified sediment samples were extracted and analyzed by LC/MS/MS as described below (Attachment I, p. A18).

6. Sampling:

Table 6: Sampling details.

Criteria		**Details**
Sampling intervals (posttreatment)	Water (clothianidin):	2, 4, 7, 14, 28, 42, 56, 70, 84 and 98 days (Table 5, p. 44).
	Sediment (clothianidin):	7, 14, 28, 42, 56, 70, 84 and 98 days.
	Phytoplankton (water):	0, 7, 14, 21, 28, 42, 56, 70, 84 and 98 days.
	Zooplankton (water):	0, 2, 4, 7, 14, 21, 28, 42, 56, 70, 84 and 98 days.
	Macrozoobenthos[1] (sediment):	0, 7, 14, 28, 42, 56, 70, 84 and 98 days.

Table 6: Sampling details.

Criteria		Details
	Emergence traps:	0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98 days.
	MASS units:	0, 14, 28, 42, 56, 70, 84 and 98 days.
Sampling method	Water:	Water samples were withdrawn using an electric vacuum (Elektrostar, Type Starmix Zyklon HG-81) equipped with a suction tube (diameter *ca*. 50 mm) and 15-L collection tank (p. 43). Six water samples (*ca*. 10 L) were taken from fixed locations in each pond system; three at *ca*. 10-20 cm from the pond tank wall and three at *ca*. 40-60 cm from the wall, with the same sampling locations used at each collection interval. The six water samples per pond system were combined in a round 80-L bucket, thoroughly mixed, then subsamples were taken as follows: clothianidin analysis - triplicate 100-mL aliquots (p. 54), phytoplankton (taxonomy and counts) - *ca*. 250 mL (p. 45), phytoplankton (pigment analyses) - 0.4-3.57 L (p. 45), and zooplankton (taxonomy and counts) - 3.5-14.3 L (p. 46). Following sub-sampling, the remainder of the water sample was returned to the respective pond (p. 43).
	Sediment:	Single or duplicate samples were collected in each pond system using an Ekman sediment sampler (15 x 15 cm, 225 cm^2 sampling area) within a zone between 10-15 cm and 50-60 cm from the pond tank wall (pp. 48, 54; Figure 6, p. 50). For each collection, the open sampler was inserted *ca*. 10-15 cm into the sediment, the sampler scoop was closed, then the sampler was slowly withdrawn and the sediment placed in a bucket for transport; sediment sample volume was *ca*. 2-3 L (p. 48). Each sediment sample was collected in a new, undisturbed location within the pond system (p. 48).
	Emergence traps:	Insects, in preservation solution, were collected from the emergence traps at each sampling interval; no additional details were provided (p. 47).
	MASS units:	At each sampling interval, MASS units were removed from each pond, then debris and colonizing organisms were washed off of the units under tap water (p. 48). Cleaned MASS units were returned to the respective pond the same sampling day.
Sampling intervals/times (posttreatment) for system parameters in the water layer:		(Attachment II, Tables 94-110, pp. B58-B64).
Conductivity, pH, temperature:		0, 2, 4, 7, 14, 21, 28, 42, 56, 70, 84 and 98 days. Water temperature was measured at *ca*. 0.6 m depth (Figure 8, p. 66).
Dissolved oxygen:		0, 2, 4, 7, 14, 28, 42, 56, 70, 84 and 98 days.
Hardness, ammonium, nitrite, nitrate, total nitrogen, dissolved ortho-phosphate, total phosphorus and total organic carbon:		0, 14, 42, 70 and 98 days.
Water level:		0, 2, 4, 7, 14, 21, 28, 42, 49, 56, 70, 84 and 98 days.
Diurnal measurements for conductivity, dissolved oxygen, pH and temperature:		At 6, 9, 12, 15 18 and 21 hours on days 7, 56 and 98.
Sample storage before analysis (storage intervals were not provided)	Water and sediment for clothianidin analyses:	Immediately after collection, the samples were stored deep-frozen (≤delete space -20ºC) in tightly sealed flasks until processing and analysis (p. 55).
	Phytoplankton, zooplankton (taxonomy and counts):	Following preservation (see **Extraction/clean up/concentration methods** below), samples were stored at room temperature in darkness (pp. 45-46).

Table 6: Sampling details.

Criteria		Details
	Phytoplankton (pigment analyses):	Not reported.
	Benthic macroinvertebrates from sediment and MASS units:	Following isolation and preservation, (see **Extraction/clean up/concentration methods** below), samples were stored at room temperature in darkness (p. 48).
	Emergent insects:	Preservation solution (recovered from the traps) containing the insects was stored at room temperature in darkness (p. 47)
Other observations, if any	Periphyton:	Clothianidin was not expected to have any relevant toxic effect on periphyton algae; therefore, periphyton growth was not monitored in detail (p. 46). However, intense growth of periphyton from the pond tank walls extending into the body of the water occurred in some of the pond systems during June and July, 2000. The dense periphyton growth (filaments/wool) interfered with water sampling, consequently, periphyton was periodically removed (days 28, 42, 49, 56, 68, 84 and 98 posttreatment) using a rake and the dry weight biomass determined (p. 46; Table 20, p. 226).
	Floating macrophytes (*Lemna sp.*):	Similarly, clothianidin was not expected to have any relevant toxic effect on floating macrophytes; however, intense growth of *Lemna sp.* occurred in most of the pond systems during late May until study termination (August, 2000; p. 46). Consequently, floating macrophytes were removed (same intervals as periphyton) when the plants covered the pond surface area by >2-5% (600-1,900 cm^2) and the dry weight biomass determined (p. 46; Table 20, p. 226).

1 Benthic macroinvertebrates (p. 47).
Data were obtained from p. 43; Table 5, p. 44; pp. 45-48; Figure 6, p. 50; pp. 54-55; Figure 8, p. 66; Table 20, p. 226; Attachment II, Tables 94-110, pp. B58-B64 of the study report.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: Water samples (100 mL) from ponds nominally treated at 10 µg a.i./L (20 µg formulation/L, days 2-28) and 3.1 µg a.i./L (6.3 µg formulation/L, days 2-4) were thawed and mechanically shaken (mechanism, speed, interval not reported), then analyzed directly by HPLC/UV as described below (p. 54; Attachment I, p. A10). All other water samples (100 mL) were thawed and applied to a solid-phase extraction (SPE) cartridge (C-18, Varian No. 12102052) preconditioned with methanol (20 mL) followed by water (20 mL x 2; Attachment I, pp. A7, A10). Clothianidin residues were eluted with methanol (20 mL), then the eluate was taken to dryness via rotary evaporation (temperature not reported) and resulting residues reconstituted in water for HPLC/UV or LC/MS/MS analyses (Attachment I, p. A10).

Sediment samples (200 mL) were thawed and centrifuged (10,000 rpm, 30 minutes), with the aqueous phase discarded (p. 54; Attachment I, pp. A17-A18). An aliquot (20 g wet wt., *ca*. 13 g dry wt.) was extracted once with water:acetonitrile:acetic acid (800:200:0.8, v:v) via shaking (mechanism, speed not reported) for 30 minutes (Attachment I, pp. A17-A18, A28). The sediment extract was filtered (filter type not specified), with the filter residue washed once with the extraction solvent (10 mL). The sample was brought to volume (50 mL) and further filtered (0.45-µm) prior to LC/MS/MS analysis. Only sediment samples from the ponds treated at 3.1 µg

a.i./L (7-56 days) and 10 µg a.i./L (all intervals) were analyzed; for all other sediment samples, the levels of parent clothianidin were expected to be below the method limit of detection (p. 55).

Phytoplankton (taxonomy and counts): Water samples (*ca*. 250 mL) were preserved with Lugol's solution (*ca*. 3 mL, p. 45).

Phytoplankton (chlorophyll-a and phaeopigments): Water samples (0.4-3.57 L) were sieved (365-µm) to remove large zooplankton organisms, then filtered through a glass microfiber filter (Whatman GF/C, p. 45). The glass filter was immersed in boiling 90% ethanol (10 mL) in a centrifuge tube containing small glass beads (p. 45). The glass filter was then mechanically ground using a spatula. Once ground, the centrifuge was sealed and maintained overnight at room temperature in darkness. The next day, the sample was brought to volume with ethanol and filtered (paper filter). Filtrates were analyzed prior to and after acidification using UV detection; acidification step was not further described.

Zooplankton (taxonomy and counts): Water samples (3.5-14.3 L) were sieved (55-µm), then organisms retained in the sieve residue were fixed in 95% ethanol followed by preservation in 70% ethanol:40 g/L saccharose:40 ml/L glycerine (p. 46).

Benthic macroinvertebrates (macrozoobenthos): Benthic organisms were recovered from sediment samples (from upper layer *ca*. 2-4 cm, *ca*. 500 mL) by washing the sediment through a kitchen sieve (*ca*. 1-mm mesh) using tap water (p. 48). Benthic macroinvertebrates were similarly recovered from the MASS units by washing the organisms off the units under tap water with collection in a kitchen sieve (1.5-mm, p. 48). Organisms retained in the sieve residues were preserved in 70% ethanol:40 g/L saccharose:40 ml/L glycerine (p. 48).

Periphyton and macrophytes (*Lemna sp.*) were dried at *ca*. 80ºC for 1-3 days for biomass determinations (p. 46).

Determination of nonextractable residues: Not applicable.

Derivatization method, if used: None was reported.

Identification and quantification of parent compound: Water samples from ponds nominally treated at 10 µg a.i./L (20 µg formulation/L, all intervals), 3.1 µg a.i./L (6.3 µg formulation/L, all intervals) and 1.0 µg a.i./L (2.0 µg formulation/L, 2-28 days) were analyzed using reverse-phase HPLC/UV under the following conditions: Purospher STAR RP-18e column (4 x 55 mm, 3 µm), gradient mobile phase combining (A) 0.1% aqueous acetic acid and (B) acetonitrile [percent A:B at 0-1 min. 90:10 (v:v), 10-15 min. 20:80, 15.1-22 min. 90:10], ambient temperature, injection volume 200 µL, flow rate 1.0 mL/minute, UV detector (264 nm), clothianidin retention time *ca*. 4.3-5.2 min. (Attachment I, pp. A10-A11, A13). Parent clothianidin was identified by comparison to the retention time of reference standard (Attachment I, Figures 1-2, pp. A44; Figures 5-11, pp. A46-A50).

All other water samples were analyzed by LC/MS/MS under the following conditions: Phenomenex Hypersil C18 BDS LC column (4.6 x 100 mm, 3 µm), gradient mobile phase combining (A) 0.1% aqueous acetic acid and (B) acetonitrile [percent A:B at 0-1 min. 90:10 (v:v), 8-13 min. 20:80, 13.1-20 min. 90:10], injection volume 70 µL, flow rate 1.0 mL/minute, clothianidin retention time *ca*. 5.3-5.8 min., Finnigan TSQ MS/MS, electrospray ionization (ESI), positive ion mode, capillary voltage 11.7 V, discharge current *ca*. 3.5 µA, spray voltage *ca*. 4.5 kV, capillary temperature 300ºC, sheath gas 70 psi N_2, auxiliary gas 3 psi N_2, detection interval 1.9-9.5 min., parent mass 250.2, fragment 1 168.6, fragment 2 131.7 (used for detection), scan time 0.5 sec. (Attachment I, p. A14). Identification of clothianidin in the samples was made against reference standard (Figures 12-17, pp. A51-A53). Results from the LC/MS/MS analyzed pond water samples were corrected by a mean recovery of 64% obtained during the method validation (see **D. SUPPLEMENTARY EXPERIMENT-RESULTS** below; Attachment I, p. A24).

All sediment extract samples were analyzed by LC/MS/MS as described above with the following modifications: Purospher STAR RP-18e column (4 x 55 mm, 3 µm), gradient mobile phase combining (A) 0.1% aqueous formic acid and (B) 0.1% formic acid in methanol [percent A:B at 0-1 min. 80:20 (v:v), 6-10 min. 20:80, 10.1-15 min. 80:20], injection volume 40 µL, clothianidin retention time *ca*. 4-5 min., MS/MS parent mass 250, fragment 1 131.8 (used for detection), deuterated parent mass 253 and fragment 1 131.8 (used for detection), scan time 0.3 sec. (Attachment I, p. A20; Figures 19-25, pp. A55-A58).

Identification and quantification of transformation products: Not performed.

Table 7: Reference compounds available for identifying transformation products of clothianidin (TI-435).

Applicant's code	Chemical Name	Purity (%)	Lot/Batch No.
--[1]	--	--	--

1 Transformation products were not identified.

Detection limits (LOD, LOQ) for the parent compound: Limits of quantitation (LOQ) for clothianidin using HPLC/UV were reported as 2 µg a.i./L (4 µg TI-435 50WG/L) for water samples analyzed directly and 0.1 µg a.i./L (0.2 µg TI-435 50WG/L) for SPE-concentrated water samples (Attachment I, pp. A22-A23). For LC/MS/MS analysis of water samples limits of detection (LOD) and quantitation (LOQ) were reported as 0.015 and 0.025 µg a.i./L, respectively (0.03 µg/L and 0.05 µg/L, respectively; Attachment I, pp. A22-A23). For LC/MS/MS analysis of sediment extracts LOD and LOQ were reported as *ca*. 4 and 8 µg a.i./kg, respectively (8 and 15.6 µg/kg TI-435 50WG/kg, respectively; Attachment I, p. A28).

Organism taxonomic identification and population counts: Phytoplankton: Preserved samples were shaken to resuspend the algal cells, then an aliquot was transferred into a 25-mL settling chamber and allowed to sit overnight (p. 45). Phytoplankton were then identified (genus

or species) using an inverted microscope (Leitz 'Diavert', 400x magnification) and systematic keys (Huber-Pestalozzi, 1962-1983; p. 45). Additional unpreserved phytoplankton samples were also used for taxonomic identification (p. 45). The density of the total taxa were quantified by a counting a sufficient number of phytoplankton using the inverted microscope, and population densities were determined from the count numbers and volume of the settling chamber, then reported as cells per water volume (p. 45).

Zooplankton: Preserved samples (entire or subsample) were quantitatively transferred to a counting dish, then identified (genus or species) using systematic keys (Einsle, 1993; Flößner, 2000; Keifer, 1973, Koste, 1978; p. 46). Numbers of individual taxon were determined using an inverted microscope (Leitz, 'Diavert') and reported as individuals per water volume (p. 46).

Benthic macroinvertebrates: Preserved samples were transferred to a counting dish, then identified (genus or species) using systematic keys (Bayerisches Landesamt für Wasserwirtschaft, 1992; Brinkhurst, 1971; Brohmer, 1971; Gloer und Meier-Brook, 1998; Stresemann, 1978; p. 48). Numbers of individual taxon were determined using a dissecting microscope (6-50x magnification) and reported as individuals per MASS unit or sediment sample (p. 46).

Emergent insects: Preserved samples were transferred to a counting dish, then identified (genus or species) using systematic keys (Bauernfeind, 1994; Pinder, 1978; Stresemann, 1978; Wiederholm, 1989; p. 47). Numbers of individual taxon were determined using a dissecting microscope (6-50x magnification) and reported as individuals per emergence trap and week (p. 47).

Determination of phytoplankton chlorophyll-a and phaeopigments (phaeophytin):
Phytoplankton extracts were analyzed for chlorophyll-a using UV detection (665 nm and 750 nm, Hitachi 100-40), then extracts were acidified (method not reported) and re-analyzed at 665 nm (p. 45).

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS:

Table 8: Water conditions [mean ± s.d. (range)] following spray application of clothianidin (TI-435 50WG) to simulated pond systems.[1]

Property	Treated Pond Systems	Control Pond Systems
Temperature (°C)	17.0 ± 3.0 (13.1-22.7)	16.9 ± 2.9 (13.1-22.2)
pH	7.8 ± 0.7 (7.1-10.0)	7.8 ± 0.6 (7.1-9.9)
Oxygen concentration (mg/L)	7.2 ± 3.7 (0.9-19.0)	7.2 ± 3.6 (1.9-15.2)
Total organic carbon (mg/L)	17.2 ± 4.6 (11.6-35.1)	17.7 ± 7.5 (11.4-43.2)
Hardness (mmol/L)	0.97 ± 0.06 (0.80-1.09)	0.98 ± 0.08 (0.71-1.07)
Electrical conductivity (µS/cm)	252 ± 16 (200-280)	256 ± 22 (186-284)
Ammonium (NH_4^+, mg N/L)	1.21 ± 1.17 (0.00-3.49)	1.42 ± 1.22 (0.03-3.55)
Nitrite (NO_2^-, mg N/L)	0.25 ± 0.30 (0.00-1.24)	0.25 ± 0.28 (0.02-1.09)
Nitrate (NO_3^-, mg N/L)	0.47 ± 0.29 (0.04-1.13)	0.54 ± 0.25 (0.16-1.19)
Total nitrogen (mg N/L)	3.65 ± 1.33 (1.57-5.54)	3.82 ± 1.36 (1.95-5.98)
Dissolved ortho-phosphate (H_3PO_4, mg P/L)	0.16 ± 0.08 (0.05-0.47)	0.18 ± 0.09 (0.05-0.34)
Total phosphorous (mg P/L)	0.36 ± 0.16 (0.14-0.90)	0.40 ± 0.19 (0.15-0.80)
Water level (cm)	110.8 ± 4.3 (102.5-126.2)	110.6 ± 4.0 (102.8-121.0)

1 Means and standard deviations calculated by the primary reviewer using data obtained from Attachment II, Tables 94-106, pp. B58-B62 of the study report (Reviewer's Comment No. 1, DER Attachment 2).

In the treated pond systems, mean (n = 10) water temperature increased from 14.3 ± 0.1ºC (range 14.2-14.5ºC) at day 0 posttreatment to 20.1 ± 0.4ºC (range 19.3-20.5ºC) at study termination (98 days; Figure 8, pp. 66-67; DER Attachment 2). Pond water levels were adjusted just prior to treatment, then remained at relatively steady levels, without any water additions, throughout the 98-day study (pp. 36, 40, 64; Figure 7, p. 65; DER Attachment 2). Diurnal measurements in the pond water at 7, 56 and 98 days, found gradual increases in dissolved oxygen concentration, pH and temperature throughout the day, while conductivity remained relatively steady-state (DER Attachment 2). Conditions in the treated pond systems were comparable to those measured in the untreated control pond systems (DER Attachment 2).

Meteorological conditions, including air temperature, barometer reading, precipitation, relative air humidity, solar radiation, wind speed and primary wind direction, were monitored by a nearby weather station; however, the data were not provided (pp. 53, 64).

B. APPLICATION VERIFICATION: Recoveries of clothianidin in the formulated application solutions averaged 104 ± 1% (range 102-105%, n = 10) of the initial nominal concentrations (p. 62; DER Attachment 2).

For the five application rates, clothianidin was detected in the water layer at 2 days posttreatment (first sampling interval) as follows:

- 0.10 µg a.i./L rate: Clothianidin was detected at 0.0583 ± 0.0097 µg a.i./L (0.0549-0.0651 µg a.i./L), equivalent to 59 ± 5% (56-66%) of nominal applied (DER Attachment 2).
- 0.31 µg a.i./L rate: Clothianidin was detected at 0.245 ± 0.015 µg a.i./L (0.238-0.252 µg a.i./L), equivalent to 79 ± 2% (76-81%) of nominal applied (DER Attachment 2).
- 1.0 µg a.i./L rate: Clothianidin was detected at 0.92 ± 0.25 µg a.i./L (0.80-1.04 µg a.i./L), equivalent to 94 ± 12% (81-106%) of nominal applied.
- 3.1 µg a.i./L rate: Clothianidin was detected at 2.92 ± 0.11 µg a.i./L (2.86-2.98 µg a.i./L), equivalent to 94 ± 2% (92-96%) of nominal applied.
- 10 µg a.i./L rate: Clothianidin was detected at 9.88 ± 0.17 µg a.i./L (9.80-9.97 µg a.i./L), equivalent to 100 ± 1% (99-101%) of nominal applied.

Table 9: Dissipation of clothianidin (TI-435 50WG), expressed as concentration (µg a.i./L for water, µg a.i./kg for sediment; mean ± s.d., n = 2-4[1]), following spray application to simulated pond systems.

Nominal rate applied (µg a.i./L)		**Sampling times (days posttreatment)**									
		2	**4**	**7**	**14**	**28**	**42**	**56**	**70**	**84**	**98**
0.10	Water	0.0583 ± 0.0097 (59 ± 5)[2]	0.0551 ± 0.0171 (56 ± 9)	0.0481 ± 0.0006 (49 ± 0)	0.0360 ± 0.0012 (37 ± 1)	0.0266 ± 0.0049 (27 ± 2)	0.0187 ± 0.0067 (19 ± 3)	<LOD	<LOD	<LOD	<LOD
	Sediment	NA[3]	NA	NA	NA	NA	NA	NA	NA	NA	NA
0.31	Water	0.245 ± 0.015 (79 ± 2)	0.207 ± 0.031 (66 ± 5)	0.141 ± 0.002 (45 ± 0)	0.133 ± 0.023 (43 ± 4)	0.0679 ± 0.0028 (22 ± 0)	0.0547 ± 0.0050 (18 ± 1)	0.0331 ± 0.0120 (11 ± 2)	0.0251 ± 0.0029 (8 ± 0)	0.0182 ± 0.0095 (6 ± 2)	0.0183 ± 0.0099 (6 ± 2)
	Sediment	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1.0	Water	0.92 ± 0.25 (94 ± 12)	0.69 ± 0.01 (70 ± 0)	0.67 ± 0.01 (68 ± 1)	0.455 ± 0.228 (46 ± 11)	0.367 ± 0.036 (37 ± 2)	0.192 ± 0.013 (19 ± 1)	0.119 ± 0.002 (12 ± 0)	0.090 ± 0.004 (9 ± 0)	0.064 ± 0.019 (7 ± 1)	0.047 ± 0.011 (5 ± 1)
	Sediment	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3.1	Water	2.92 ± 0.11 (94 ± 2)	2.76 ± 0.10 (89 ± 2)	2.31 ± 0.06 (74 ± 1)	2.20 ± 0.08 (71 ± 1)	1.32 ± 0.03 (42 ± 0)	0.99 ± 0.13 (32 ± 2)	0.67 ± 0.03 (22 ± 0)	0.541 ± 0.133 (17 ± 2)	0.438 ± 0.086 (14 ± 1)	0.295 ± 0.071 (9 ± 1)
	Sediment	NA	NA	<LOD	<LOD	5.0 ± 1.4	4.3 ± 0.3	2.6 ± 2.1	NA	NA	NA
10	Water	9.88 ± 0.17 (100 ± 1)	9.45 ± 1.12 (96 ± 6)	8.11 ± 0.37 (82 ± 2)	5.29 ± 0.64 (54 ± 3)	4.67 ± 0.29 (47 ± 1)	3.10 ± 0.29 (31 ± 1)	2.18 ± 0.25 (22 ± 1)	1.48 ± 0.20 (15 ± 1)	1.02 ± 0.33 (10 ± 2)	0.76 ± 0.07 (8 ± 0)
	Sediment	NA	NA	12.6 ± 5.8	5.8 ± 2.9	15.2 ± 13.2	12.5 ± 1.3	9.6 ± 2.0	8.8 ± 2.5	6.9 ± 2.7	4.2 ± 2.6

1 Means and standard deviations calculated by the primary reviewer using data obtained from Attachment I, Table 4, pp. A36-A38; Table 8, pp. A42-A43 of the study report (Reviewer's Comment No. 1, DER Attachment 2).

2 Results in parentheses = percent of nominal applied.

3 Not analyzed.

C. RESIDUE ANALYSIS:

1. Water: 0.10 µg a.i./L rate: Clothianidin residues in the water layer decreased from 0.0549-0.0651 µg a.i./L (56-66% of nominal applied) at 2 days posttreatment (first interval analyzed) to 0.0478-0.0484 µg a.i./L (49%) at 7 days, 0.0242-0.0290 µg a.i./L (25-29%) at 28 days, 0.0154-0.0219 µg a.i./L (16-22%) at 42 days and were <LOD at 56-98 days (DER Attachment 2).

0.31 µg a.i./L rate: Clothianidin residues in the water decreased from 0.238-0.252 µg a.i./L (76-81% of applied) at 2 days to 0.140-0.141 µg a.i./L (45%) at 7 days, 0.0666-0.0693 µg a.i./L (21-22%) at 28 days, 0.0272-0.0389 µg a.i./L (9-13%) at 56 days and were 0.0134-0.0232 µg a.i./L (4-7%) at 98 days.

1.0 µg a.i./L rate: Clothianidin residues in the water decreased from 0.80-1.04 µg a.i./L (81-106% of applied) at 2 days to 0.342-0.57 µg a.i./L (35-58%) at 14 days, 0.185-0.198 µg a.i./L (19-20%) at 42 days, 0.089-0.092 µg a.i./L (9%) at 70 days and were 0.041-0.052 µg a.i./L (4-5%) at 98 days.

3.1 µg a.i./L rate: Clothianidin residues in the water decreased from 2.86-2.98 µg a.i./L (92-96% of applied) at 2 days to 2.16-2.24 µg a.i./L (70-72%) at 14 days, 1.30-1.33 µg a.i./L (42-43%) at 28 days, 0.66-0.69 µg a.i./L (21-22%) at 56 days and were 0.260-0.330 µg a.i./L (8-11%) at 98 days.

10 µg a.i./L rate: Clothianidin residues in the water decreased from 9.80-9.97 µg a.i./L (99-101% of applied) at 2 days to 4.98-5.61 µg a.i./L (51-57%) at 14 days, 4.53-4.81 µg a.i./L (46-49%) at 28 days, 2.05-2.30 µg a.i./L (21-23%) at 56 days, 0.86-1.19 µg a.i./L (9-12%) at 84 days and were 0.73-0.79 µg a.i./L (7-8%) at 98 days.

2. Sediment: Sediment from ponds treated at 0.10, 0.31 and 1.0 µg a.i./L were not analyzed for clothianidin.

3.1 µg a.i./L rate: Clothianidin residues were initially detected at 4.4-6.2 µg a.i./kg at 28 days decreasing to 2.0-4.3 µg a.i./kg at 56 days; sediment collected at 70-98 days was not analyzed (DER Attachment 2).

10 µg a.i./L rate: Clothianidin residues were detected at 9.7-16.0 µg a.i./kg at 7 days (first interval analyzed), were a maximum 22.0 µg a.i./kg at 28 days, then decreased to 8.1-10.9 µg a.i./kg at 56 days and were 2.0-5.1 µg a.i./kg at 98 days.

Half-life/DT50/DT90: Observed DT50 values for clothianidin in the water from the 0.10, 0.31, 1.0, 3.1 and 10 µg a.i./L-treated ponds were *ca*. 7, 6, 13, 24 and 22 days, respectively, with respective DT90 values of *ca*. 49, 61, 65, 95 and 84 days. First-order linear regression analysis (Excel 2007; 0-42 days for 0.10 µg a.i./L ponds, all intervals for remaining ponds) yielded half-lives for clothianidin in the water from the 0.10, 0.31, 1.0, 3.1 and 10 µg/L-treated ponds of 22,

24, 23, 29 and 26 days, respectively, with respective nonlinear (SigmaPlot v 9.0) half-lives of 17, 15, 18, 27 and 24 days (DER Attachment 2).

In sediment from the 3.1 and 10 µg a.i./L-treated ponds, observed DT50 values for clothianidin were *ca*. 56 and 79 days, respectively, with respective DT90 values of >56 and >98 days. First-order linear and nonlinear analyses determined half-lives for clothianidin in the sediment of 27 and 33 days, respectively, for the 3.1 µg a.i./L-treated ponds (28-56 days) and half-lives of 42 and 45 days, respectively, for the 10 µg a.i./L-treated ponds (28-98 days, DER Attachment 2).

Half-lives/DT50/DT90 for clothianidin (TI-435) in water and sediment:

Application	Half-life/DT50[1] (days)	First-order linear regression equation	R^2	Observed DT50[2] (days)	Observed DT90[2] (days)
0.10 µg a.i./L Treatment Rate					
Water					
Linear/natural log	22.4	y = -0.0310x - 2.7599	0.8705	*ca*. 7	*ca*. 49
Nonlinear/normal	17.4	y = 0.0685exp(-0.0398x)	0.9629		
Sediment					
Linear/natural log	NA[3]	--	--	--	--
Nonlinear/normal	NA	--	--		
0.31 µg a.i./L Treatment Rate					
Water					
Linear/natural log	24.2	y = -0.0286x - 1.5943	0.9346	*ca*. 6	*ca*. 61
Nonlinear/normal	14.6	y = 0.2591exp(-0.0474x)	0.9734		
Sediment					
Linear/natural log	NA	--	--	--	--
Nonlinear/normal	NA	--	--		
1.0 µg a.i./L Treatment Rate					
Water					
Linear/natural log	22.5	y = -0.0308x - 0.2152	0.9743	*ca*. 13	*ca*. 65
Nonlinear/normal	18.3	y = 0.9022exp(-0.0378x)	0.9786		
Sediment					
Linear/natural log	NA	--	--	--	--
Nonlinear/normal	NA	--	--		
3.1 µg a.i./L Treatment Rate					
Water					
Linear/natural log	29.2	y = -0.0237x + 1.0549	0.9840	*ca*. 24	*ca*. 95
Nonlinear/normal	26.5	y = 3.0182exp(-0.0262x)	0.9967		
Sediment					
Linear/natural log	27.0	y = -0.0257x + 2.3882	0.6089	*ca*. 56	>56
Nonlinear/normal	33.0	y = 9.2527exp(-0.0210x)	0.9635		
10 µg a.i./L Treatment Rate					

Half-lives/DT50/DT90 for clothianidin (TI-435) in water and sediment:

Application	Half-life/DT50[1] (days)	First-order linear regression equation	R^2	Observed DT50[2] (days)	Observed DT90[2] (days)
Water					
Linear/natural log	26.2	y = -0.0265x + 2.2608	0.9860	*ca*. 22	*ca*. 84
Nonlinear/normal	23.8	y = 10.0419exp(-0.0291x)	0.9916		
Sediment					
Linear/natural log	41.5	y = -0.0167x + 3.1956	0.6470	*ca*. 79	>98
Nonlinear/normal	44.7	y = 23.8047exp(-0.0155x)	0.9244		

1 Determined by the primary reviewer using Excel 2007 (linear, first-order) and SigmaPlot v 9.0 (nonlinear, one-compartment/two-parameter) and individual sample data obtained from Attachment I, Table 4, pp. A36-A38; Table 8, pp. A42-A43 of the study report (DER Attachment 2).

2 Observed DT50 and DT90 values were determined by the primary reviewer using mean clothianidin detected at each interval and assumed 100% application to water layer at time 0 (DER Attachment 2).

3 Not analyzed.

Table 10: Effects of clothianidin (TI-435 50WG) on phytoplankton, expressed as concentration (mean ± s.d., n = 2, except untreated controls n = 3[1]), following spray application to simulated pond systems.[2]

Nominal rate applied (µg a.i./L)	Sampling times (days posttreatment)									
	0	**7**	**14**	**21**	**28**	**42**	**56**	**70**	**84**	**98**
	Total Phytoplankton (cells/mL)									
0.10	258.58 ± 15.17	1,342.56 ± 84.76	16,940.94 ± 15,136.81	7,868.47 ± 4,511.79	5,442.03 ± 4,456.25	2,683.87 ± 1,564.85	4,484.10 ± 3,659.24	2,140.22 ± 1,662.02	4,642.30 ± 3,579.70	2,024.61 ± 1,232.72
0.31	348.62 ± 8.52	1,026.26 ± 637.85	1,663.27 ± 887.48	1,710.14 ± 500.50	6,010.83 ± 3,192.86	558.13 ± 152.63	623.73 ± 349.68	1,159.09 ± 527.46	718.19 ± 177.43	9,196.22 ± 7,989.28
1.0	225.06 ± 29.65	781.45 ± 361.89	1,090.73 ± 811.03	2,338.53 ± 1,397.18	1,957.84 ± 365.01	12,339.99 ± 10,775.36	338.88 ± 170.53	643.73 ± 190.92	756.20 ± 2.26	1,169.28 ± 505.33
3.1	240.94 ± 156.44	391.09 ± 211.54	5,218.96 ± 3,783.62	4,365.80 ± 947.28	294,016.54 ± 292,482.57	2,128.55 ± 186.64	1,163.49 ± 757.91	596.81 ± 455.07	5,027.28 ± 4,033.86	5,883.26 ± 2,606.82
10	220.36 ± 48.07	1,386.14 ± 321.68	5,247.62 ± 3,635.45	11,939.79 ± 9,663.70	1,377.49 ± 121.18	6,225.59 ± 4,902.44	673.75 ± 501.48	2,480.76 ± 2,082.91	233.79 ± 67.98	1,109.96 ± 257.20
Controls	179.20 ± 35.05	731.48 ± 152.54	1,663.27 ± 45.96	9,840.62 ± 5,898.04	4,859.81 ± 3,346.08	6,321.13 ± 7,081.38	651.49 ± 344.09	742.10 ± 458.11	1,210.33 ± 213.17	2,528.88 ± 941.10
	Chlorophyll-a (µg/L)									
0.10	0.70 ± 0.19	2.34 ± 0.27	ND[3], 18.35	46.72 ± 41.79	30.07 ± 27.55	9.02 ± 4.88	24.79 ± 23.16	22.43 ± 19.32	21.76 ± 15.25	10.95 ± 7.99
0.31	0.89 ± 0.48	0.74 ± 0.64	ND	4.64 ± 2.67	3.99 ± 1.77	5.63 ± 0.60	1.78 ± 0.00	4.99 ± 1.73	8.64 ± 5.28	3.46 ± 0.89
1.0	1.45 ± 0.10	1.09 ± 0.60	ND	2.59 ± 0.22	1.41 ± 0.37	9.13 ± 3.51	4.05 ± 3.66	7.38 ± 5.64	3.83 ± 1.94	6.51 ± 2.07
3.1	3.00 ± 1.87	1.23 ± 0.05	4.15 ± 3.85	4.96 ± 1.56	6.39 ± 5.06	39.99 ± 35.70	10.49 ± 7.28	0.86 ± 0.18	17.23 ± 12.79	11.55 ± 2.37
10	1.85 ± 0.07	1.58 ± 0.29	ND, 18.35	11.47 ± 8.36	3.41 ± 1.34	18.56 ± 14.42	1.29 ± 0.30	21.02 ± 4.44	10.73 ± 7.33	14.80 ± 2.22
Controls	1.14 ± 0.80	0.63 ± 0.25	1.94 ± 1.53	9.86 ± 6.78	7.69 ± 5.99	7.00 ± 3.00	5.43 ± 5.52	5.06 ± 5.21	22.74 ± 17.78	41.05 ± 16.58
	Phaeophytin (µg/L)									
0.10	1.62 ± 0.01	1.67, ND	17.41 ± 11.26	7.54 ± 0.16	15.01 ± 12.43	13.77 ± 10.26	31.58 ± 29.05	3.94 ± 0.29	14.13 ± 12.32	6.32 ± 2.62
0.31	2.10 ± 0.69	ND, 1.01	6.88 ± 1.20	4.96, ND	3.72 ± 1.16	8.21 ± 2.57	6.44 ± 4.47	3.63 ± 2.83	7.69 ± 5.85	2.65 ± 0.36
1.0	2.31 ± 0.22	1.31 ± 0.28	5.71 ± 0.67	2.49, ND	1.97 ± 0.11	8.23 ± 0.20	8.71 ± 4.81	ND, 9.64	4.20 ± 2.93	4.51 ± 0.63
3.1	1.34, ND	1.40 ± 0.09	11.14 ± 4.16	ND, 5.00	5.26 ± 3.68	48.91 ± 42.39	13.37 ± 9.64	5.33 ± 0.08	6.98 ± 1.64	5.51 ± 1.12
10	1.12 ± 0.19	0.99 ± 0.08	4.93 ± 0.18	3.46 ± 2.51	4.33 ± 2.03	25.90 ± 19.56	4.13 ± 0.99	17.36 ± 0.03	5.86 ± 4.58	11.07 ± 4.95

Controls	1.26 ± 0.12	1.40 ± 0.61	4.74 ± 0.72	5.40 ± 3.67	4.40 ± 2.47	14.21 ± 1.97	12.63 ± 12.78	6.53 ± 4.63	21.22 ± 22.96	20.31 ± 10.08

1 Means and standard deviations calculated by the primary reviewer using data obtained from Attachment II, Tables 55-56, p. B38; Tables 58-74, pp. B39-B47; Tables 76-77, pp. B48-B49; Tables 79-82, pp. B50-B51; Tables 84-87, pp. B52-B54; Table 89, p. B55; Table 91, p. B56; Table 93, p. B57 of the study report (Reviewer's Comment No. 1, DER Attachment 2).

2 Results (mean ± s.d.) for individual phytoplankton taxa and phaeophytin (chlorophyll-a degradation product following acidification) are presented in DER Attachment 2.

3 Not detected.

Table 11: Effects of clothianidin (TI-435 50WG) on zooplankton, expressed as concentration (mean ± s.d., n = 2, except untreated controls n = 3[1]), following spray application to simulated pond systems.[2]

Nominal rate applied (µg a.i./L)	**Sampling times (days posttreatment)**											
	0	**2**	**4**	**7**	**14**	**21**	**28**	**42**	**56**	**70**	**84**	**98**
	Total Zooplankton (individuals/L)											
0.10	674.45 ± 159.55	352.82 ± 53.85	276.66 ± 46.20	241.79 ± 29.12	190.28 ± 59.52	669.86 ± 294.32	701.46 ± 25.89	1,286.16 ± 126.18	1,590.07 ± 1,064.48	439.44 ± 63.71	463.37 ± 167.97	616.99 ± 214.45
0.31	575.24 ± 67.95	366.35 ± 15.06	284.39 ± 14.89	298.95 ± 9.82	222.71 ± 68.53	316.24 ± 128.88	319.49 ± 128.61	196.13 ± 89.92	401.24 ± 282.93	216.73 ± 40.82	382.27 ± 179.92	397.60 ± 241.34
1.0	666.69 ± 84.68	323.03 ± 139.30	205.00 ± 75.01	316.75 ± 39.54	285.51 ± 64.49	262.09 ± 3.74	268.77 ± 16.84	296.06 ± 102.72	588.36 ± 575.90	235.54 ± 209.89	412.41 ± 143.88	562.76 ± 129.16
3.1	702.17 ± 19.54	426.75 ± 102.09	323.23 ± 100.96	260.35 ± 101.25	164.04 ± 43.58	366.72 ± 53.50	402.67 ± 117.33	2,921.98 ± 2,434.31	621.39 ± 237.00	202.64 ± 45.33	776.46 ± 315.56	984.64 ± 294.86
10	704.13 ± 58.63	361.19 ± 21.66	313.95 ± 6.04	269.42 ± 15.00	233.08 ± 34.83	532.84 ± 170.64	500.82 ± 20.82	1,607.75 ± 1,348.75	336.19 ± 5.56	553.48 ± 342.84	323.79 ± 44.39	409.67 ± 187.84
Controls	738.29 ± 41.21	351.95 ± 68.23	269.30 ± 29.58	249.94 ± 40.16	207.86 ± 100.79	300.89 ± 51.91	447.72 ± 66.43	405.25 ± 237.40	717.47 ± 322.57	151.70 ± 85.83	168.12 ± 123.62	655.14 ± 321.50

1 Means and standard deviations calculated by the primary reviewer using data obtained from Attachment II, Tables 2-6, pp. B7-B9; Tables 8-10, pp. B10-B11; Tables 12-24, pp. B12-B18 of the study report (Reviewer's Comment No. 1, DER Attachment 2).

2 Results (mean ± s.d.) for individual zooplankton taxa are presented in DER Attachment 2.

Table 12: Effects of clothianidin (TI-435 50WG) on benthic macroinvertebrates, expressed as concentration (mean ± s.d., n = 2 or 4 for treated, with untreated controls n = 3[1]), following spray application to simulated pond systems.[2]

Nominal rate applied (µg a.i./L)	Sampling times (days posttreatment)								
	0	**7**	**14**	**28**	**42**	**56**	**70**	**84**	**98**
	Total Benthic Macroinvertebrates Recovered in Macroinvertebrate Artificial Substrate Sampler (MASS) units (individuals/MASS unit, treated n = 4)								
0.10	103.3 ± 22.4	NS[3]	220.5 ± 81.4	81.8 ± 62.7	191.5 ± 163.9	253.5 ± 137.5	285.0 ± 86.0	373.0 ± 269.1	375.3 ± 259.5
0.31	104.0 ± 42.4	NS	126.0 ± 93.3	118.0 ± 55.5	144.8 ± 53.4	341.5 ± 95.6	368.3 ± 94.3	387.0 ± 199.4	390.0 ± 159.6
1.0	57.0 ± 23.8	NS	120.0 ± 97.8	63.5 ± 36.1	198.3 ± 65.1	329.0 ± 156.1	398.5 ± 194.6	408.8 ± 201.0	290.8 ± 31.3
3.1	42.3 ± 28.4	NS	124.3 ± 84.0	108.0 ± 75.3	139.0 ± 92.7	183.0 ± 126.3	297.3 ± 154.9	147.8 ± 84.7	164.5 ± 27.3
10	84.0 ± 20.5	NS	76.0 ± 22.4	76.3 ± 45.1	75.0 ± 13.8	175.0 ± 75.2	218.3 ± 15.8	140.8 ± 34.6	214.3 ± 94.8
Controls	82.0 ± 28.8	NS	191.7 ± 159.2	98.0 ± 36.0	113.3 ± 47.2	303.0 ± 90.7	252.5 ± 127.6	325.0 ± 133.0	336.2 ± 181.7
	Total Benthic Macroinvertebrates Recovered in Sediment (individuals/sediment sample, treated n = 2)								
0.10	59.5 ± 21.5	48.0 ± 22.0	51.5 ± 8.5	43.0 ± 7.0	34.0 ± 7.0	89.0 ± 14.0	82.5 ± 6.5	154.5 ± 62.5	115.0 ± 52.0
0.31	45.5 ± 17.5	44.5 ± 0.5	33.0 ± 2.0	40.5 ± 12.5	65.0 ± 45.0	101.0 ± 44.0	83.5 ± 7.5	48.5 ± 0.5	37.0 ± 10.0
1.0	22.0 ± 4.0	41.5 ± 2.5	42.0 ± 8.0	63.5 ± 10.5	38.5 ± 7.5	52.0 ± 20.0	54.0 ± 21.0	51.0 ± 1.0	38.5 ± 13.5
3.1	31.0 ± 12.0	39.5 ± 7.5	43.5 ± 9.5	40.0 ± 2.0	92.0 ± 60.0	64.0 ± 10.0	82.0 ± 8.0	71.5 ± 21.5	62.0 ± 2.0
10	51.5 ± 4.5	29.0 ± 11.0	37.0 ± 1.0	28.0 ± 2.0	35.0 ± 12.0	83.0 ± 12.0	92.0 ± 17.0	109.5 ± 76.5	116.0 ± 76.0
Controls	41.3 ± 11.6	69.7 ± 30.9	56.3 ± 7.9	46.0 ± 4.3	46.3 ± 19.3	82.3 ± 14.3	58.0 ± 17.0	68.0 ± 34.1	68.0 ± 11.8

1 Means and standard deviations calculated by the primary reviewer using data obtained from Attachment II, Tables 43-54, pp. B29-B37 of the study report (Reviewer's Comment No. 1, DER Attachment 2).

2 Results (mean ± s.d.) for individual macroinvertebrate taxa are presented in DER Attachment 2.

3 Not sampled.

Table 13: Effects of clothianidin (TI-435 50WG) on emergent insects, expressed as concentration (mean ± s.d., n = 2, except untreated controls n = 3[1]), following spray application to simulated pond systems.[2]

Nominal rate applied (µg a.i./L)	**Sampling times (days posttreatment)**														
	0	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**91**	**98**
	Total Emergent Insects (individuals/trap*week)														
0.10	7.5 ± 2.5	32.0 ± 17.0	101.0 ± 32.0	126.5 ± 13.5	95.0[3]	114.0 ± 58.0	55.5 ± 37.5	12.5 ± 7.5	74.0 ± 18.0	58.5 ± 14.5	48.0 ± 10.0	36.5 ± 4.5	49.5 ± 42.5	31.5 ± 25.5	33.0 ± 27.0
0.31	3.0 ± 0.0	12.0 ± 6.0	154.0 ± 43.0	156.0 ± 27.0	126.5 ± 10.5	194.0 ± 31.0	53.0 ± 13.0	15.0 ± 6.0	21.5 ± 14.5	27.5 ± 15.5	28.0 ± 19.0	33.5 ± 14.5	31.0 ± 3.0	43.0 ± 1.0	77.5 ± 25.5
1.0	1.5 ± 0.5	75.0 ± 72.0	76.0 ± 17.0	156.0 ± 90.0	159.5 ± 107.5	162.0 ± 76.0	51.5 ± 10.5	10.0 ± 5.0	18.5 ± 11.5	14.0 ± 3.0	27.5 ± 12.5	17.5 ± 0.5	27.5 ± 1.5	62.0 ± 3.0	167.5 ± 58.5
3.1	3.0 ± 2.0	17.0 ± 10.0	13.0 ± 9.0	28.0 ± 24.0	16.0 ± 1.0	59.0 ± 11.0	34.5 ± 12.5	11.0 ± 2.0	80.5 ± 55.5	15.0[3]	21.5 ± 3.5	19.5 ± 8.5	35.5 ± 8.5	75.0 ± 24.0	46.5 ± 2.5
10	5.0 ± 1.0	1.5 ± 0.5	ND[4], 1	ND, 2	11.5 ± 0.5	59.5 ± 6.5	27.0 ± 3.0	56.0 ± 33.0	43.5 ± 15.5	52.5 ± 3.5	26.0 ± 4.0	54.0 ± 25.0	92.0 ± 31.0	104.5 ± 85.5	213.0 ± 158.0
Controls	3.3 ± 2.9	45.3 ± 8.7	183.7 ± 112.4	145.3 ± 61.4	121.7 ± 82.1	165.3 ± 78.6	55.0 ± 38.1	48.7 ± 61.2	19.0 ± 13.5	17.7 ± 8.2	31.0 ± 16.8	33.0 ± 29.9	69.7 ± 69.6	79.3 ± 54.9	93.7 ± 83.8

1 Means and standard deviations calculated by the primary reviewer using data obtained from Attachment II, Tables 27-42, pp. B20-B28 of the study report (Reviewer's Comment No. 1, DER Attachment 2).

2 Results (mean ± s.d.) for individual insect taxa are presented in DER Attachment 2.

3 Results for single trap reported.

4 None detected.

D. ECOLOGICAL EFFECTS:

Per taxon (species up to phylum and total counts per biota type; *e.g.*, phytoplankton, zooplankton, benthic macroinvertebrates and emergent insects), univariate statistics were used to evaluate differences between organisms from the treated and control ponds and to determine no observed effect concentration (NOEC) values (pp. 56-59). EC20 and EC50 values (effective concentration for 20% and 50% inhibition, respectively) were estimated via regression analyses where instances of significant negative concentration-effect relationship (increase in negative effect with increasing test substance concentration) occurred (pp. 56, 58). Diversity (including Shannon-Weaver index), evenness, similarity (Steinhaus' and Stander's indices) and Principle Response Curve (PRC) analyses were also performed (pp. 59-61).

1. Phytoplankton: The following phytoplankton (30 taxa) were identified; approximate cell size was used for some taxa classification (p. 196; Table 18, p. 197):

Chlorophyceae
- *Ankistrodesmus acicularis*
- *Ankistrodesmus angustus*
- *Ankyra ancora*
- *Ankyra judayi*
- *Chlamydomonas sp.* (6 µm)
- *Chlamydomonas sp.* (12 µm)
- *Chlamydomonas sp.* (18 µm)
- Chlorophyte x (unknown species)
- Coccale Chlorophycea (4 µm)
- Coccale Chlorophycea (10 µm)
- *Kirchneriella sp.*
- *Lagerheimia c.f. ciliata*
- *Monoraphidium c.f. fontinalis*
- *Oocystis sp.*
- *Scenedesmus quadricaudata*
- *Schroederia sp.*
- *Volvox sp.*

Conjugatophyceae
- *Clostridium c.f. moniliferum*

Cryptophyta
- Small Cryptophytes (4 µm)
- Medium Cryptophytes (12 µm)
- Large Cryptophytes (30 µm)
- Large Cryptophytes (35 µm)

Cyanobacteria

Oscillatoria sp.

Diatomeae
Cyclotella sp.
Fragillaria sp.
Navicula sp.
Stauroneis sp.

Euglenophyta
Phacus sp.
Trachelomonas sp.

Xanthophycea
Bumilleriopsis c.f. petersiensis

There were no consistent concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L (20 µg TI-435 50WG/L), on phytoplankton taxa abundance, diversity, evenness or similarity (pp. 196, 211; Attachment II, Table 144, p. B80). Regarding taxa abundance, only at 56 days posttreatment was mean phytoplankton taxa abundance in the 10 µg a.i./L-treated ponds statistically lower than the control ponds; however, mean taxa abundance in the treated ponds then exceeded the controls at the next sampling interval at 70 days, and the decline at 56 days was considered not due to a toxic effect (p. 196; Figure 65, pp. 199-200). Regarding diversity and evenness, diversity and evenness at the highest (10 µg a.i./L) treatment rate were lower at 7 days posttreatment , as compared to the lower test concentrations; however, a pattern of decline in the 10 µg a.i./L treated ponds had begun at 7 days prior to treatment and continued up to 7 days posttreatment, and, therefore, was not considered due to a toxic effect (p. 196; Figure 66, p. 201). Similarity analyses detected a large variation between the test concentrations at 70 days posttreatment; however, no concentration-dependent trend was observed (p. 196; Figure 67, p. 202). A gradual decrease in similarity between the treated and control pond phytoplankton populations over the study period appeared due to development of different algal taxa communities (p. 204). Principle response curves of treated ponds were similar to the controls up to 7 days posttreatment; thereafter, differences between the treated and controls ponds increased, but were either not statistically significant ($p > 0.05$) or showed no concentration-dependent trend (Figure 68, p. 203; p. 204; Attachment II, Table 143, p. B80).

Concentrations of chlorophyll-a were used to estimate phytoplankton biomass and, in general, were similar between the treated and controls ponds with the exception of the Replicate B 0.1 µg a.i./L (0.2 µg TI-435 50WG/L)-treated pond (p. 205; DER Attachment II). The increased levels of chlorophyll-a in the Replicate B 0.1 µg a.i./L-treated pond were reportedly due to filamentous periphyton algae which grew on the pond walls and extended into the water sampling area (p. 205). Overall phytoplankton population densities were low for all of the ponds and presumed due to high zooplankton (grazing) populations (see below, p. 210). However, levels of chlorophyll-a and phaeophytin (chlorophyll-a degradation product) indicated clothianidin, up to 10 µg a.i./L, had no effect on phytoplankton biomass (p. 205; Figures 69-70, pp. 206-209).

Summary of effects of clothianidin (TI-435) on phytoplankton

Parameter/Taxon	NOEC[1] (µg a.i./L)	Complete Recovery (days posttreatment)
Number of taxa	10	No toxic effect.
Diversity	10	No toxic effect.
Evenness	10	No toxic effect.
Steinhaus' Similarity	10	No toxic effect.
Stander's Similarity	10	No toxic effect.
PRC[2] analysis	10	No toxic effect.
Sum of Chlorophyceae	10	No toxic effect.
Sum of Cryptophyta	10	No toxic effect.
Sum of Cyanobacteria	10	No toxic effect.
Sum of Diatomeae	10	No toxic effect.
Sum of Euglenophyta	10	No toxic effect.
Sum of Xanthophycea	10	No toxic effect.
Chlorophyll-a	10	No toxic effect.
Phaeophytin	10	No toxic effect.

1 No Observed Effect Concentration; based on Williams tests (one-sided smaller, $\alpha = 0.05$; Attachment II, Table 144, p. B80).

2 Principle Response Curve.

Data were obtained from p. 22; Attachment II, Table 144, p. B80 of the study report.

2. Zooplankton: The following zooplankton (22 taxa) were identified (p. 68; Table 6, p. 69):

Crustacea
- Cladocera
  - *Bosmina logirostris*
  - *Chydorus c.f. sphaericus*
  - *Daphnia magna*
  - *Daphnia pulex*
  - *Simocephalus vetulus*
- Copepoda
  - *Copepod nauplii*
  - Cyclopoida adults
- Ostracoda

Rotatoria
- *Brachionus angularis*
- *Brachionus bidentata*
- *Brachionus calyciflorus*
- *Brachionus urceolaris/variabilis*
- *Cephalodella sp.*
- *Colurella sp.*

Encetrum sp.
Hexarthra mira/intermedia
Keratella cochlearis
Keratella quadrata
Lecane sp.
Lepadella patella
Rotaria neptunica

There were no consistent concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L (20 µg TI-435 50WG/L), on zooplankton taxa abundance, diversity, evenness or similarity (p. 68; Attachment II, Table 117, p. B68). Regarding taxa abundance, zooplankton taxa abundance decreased in some treated ponds, as compared to the controls; however, the respective replicate treated pond was always in the range of the controls or above, and mean taxa abundance values were not significantly lower than the controls based on Williams tests (p. 68; Figure 9, pp. 70-71; Attachment II, Table 111, p. B65; Table 117, p. B68). Regarding diversity and evenness, diversity and evenness were significantly lower at the highest treatment rates (NOEC 1.0 µg a.i./L, 2.0 µg TI-435 50WG/L) at 42 days posttreatment, with diversity again significantly reduced at the 0.31-10 µg a.i./L treatment rates (NOEC 0.1 µg a.i./L, 0.2 µg TI-435 50WG/L) and evenness at all treatment rates (NOEC <0.1 µg a.i./L, <0.2 ug TI-435 50WG/L) at 84 days (p. 68; Attachment II, Tables 112-113, pp. B65-B66; Table 117, p. B68). However, there were no concentration-dependent trends for either diversity or evenness at any sampling interval; therefore, the observances at 42 and 84 days were not considered due to a toxic effect (p. 68; Figure 10, p. 72). Similarity analyses were fairly high up to 28 days posttreatment, then dropped and showed greater variation between treatment rates at 42 days (p. 73; Figure 11, p. 74; Attachment II, Tables 114-115, pp. B66-B67). However, there were no concentration-dependent trends in the similarity indices at any interval, and the decreases in the similarity indices coincided with the development of several rotifer species (*Colurella sp., Encetrum sp., Hexarthra mira/intermedia, Lecane sp., Lepadella patella* and *Rotaria neptunica*) populations from 28 days onward (p. 73; Figures 21-26, pp. 98-109). Principle response curves found no statistically significant ($p > 0.05$) differences in zooplankton community structure between the treated and control ponds, nor any concentration-dependent trends (Figure 12, p. 77; p. 78; Attachment II, Table 116, p. B67). The variations (non-significant) between treated and controls ponds that occurred from 42 days onward were presumed due to the appearance of new rotifer species as mentioned above (Figure 12, p. 77; p. 78).

Considering the individual crustacean (cladocerans, copepods and ostracods) and rotifer species, there were no concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L, on the population densities (pp. 80, 93; Figures 13-18, pp. 81-92; Figures 19-26, pp. 94-109; Attachment II, Table 117, p. B68). However, concentration-effect analyses of total cladocerans, total copepods and total rotifers found a statistically significant negative trend for total rotifers during the 70-98 day posttreatment interval, yielding EC20 and EC50 values of 9.5 µg a.i./L (18.9 µg TI-435 50WG/L) and 10.5 µg a.i./L (20.9 µg TI-435 50WG/L), respectively (p. 110; Figure 27, pp. 110-111; Table 8, p. 112).

Effective concentration values (EC20, EC50) for clothianidin (as TI-435 50WG) for zooplankton[1]

Parameter	Interval[2]	a	b	R^2	Significance	EC20		EC50	
						(µg TI-435 50WG/L)	(µg a.i./L)	(µg TI-435 50WG/L)	(µg a.i./L)
Total Cladocerans	Pre	1771.475	0.348	0.561					
	Treat			n.d.[3]					
	Post1	0.792	0.15	0.788					
	Post2			n.d.					
Total Copepods	Pre			n.d.					
	Treat			n.d.					
	Post1			n.d.					
	Post2			n.d.					
Total Rotifers	Pre			n.d.					
	Treat	24.263	10.601	0.721		(21.3)[4]	(10.7)	(24.3)	(12.2)
	Post1			n.d.					
	Post2	20.881	14.244	1.000	$p \leq 0.05$	18.9	9.5	20.9	10.5

1 Determined by study author using two-parameter nonlinear regression analysis fitted with the Downhill-simplex algorithm (pp. 56-59; Figure 27, pp. 110-111; Table 8, p. 112 of the study report).
2 Pre = pre-treatment period: -14 to 0 days,
Treat = treatment period: 2 to 14 days posttreatment,
Post1 = posttreatment period 1: 21 to 56 days posttreatment,
Post2 = posttreatment period 2: 70 to 98 days posttreatment.
3 Not determined.
4 Values in parentheses from non-significant concentration-effect relationships.
Data were obtained from Table 8, p. 112 of the study report.

Summary of effects of clothianidin (TI-435) on zooplankton

Parameter/Taxon	NOEC[1] (µg a.i./L)	Complete Recovery (days posttreatment)
Number of taxa	10	No toxic effect.
Diversity	10	No toxic effect.
Evenness	10	No toxic effect.
Steinhaus' Similarity	10	No toxic effect.
Stander's Similarity	10	No toxic effect.
PRC[2] analysis	10	No toxic effect.
Cladocera	10	No toxic effect.
Copepoda	10	No toxic effect.
Ostracoda	10	No toxic effect.
Rotatoria	10	No toxic effect.[3]

1 No Observed Effect Concentration; based on Williams tests (one-sided smaller, $\alpha = 0.05$; Attachment II, Table 117, p. B68).
2 Principle Response Curve.
3 For individual species. Nonlinear regression analyses determined EC20 and EC50 values of 9.5 µg a.i./L (18.9 µg TI-435 50WG/L) and 10.5 µg a.i./L (20.9 µg TI-435 50WG/L), respectively, for total rotifers during 70-98 day posttreatment interval (p. 110; Figure 27, p. 111; Table 8, p. 112).
Data were obtained from p. 22; Attachment II, Table 117, p. B68 of the study report.

3. Benthic macroinvertebrates recovered in MASS units: The following benthic organisms (7 taxa) were identified (p. 132; Table 12, p. 132):

Annelida
Oligochaeta
Tubificidae
Stylaria lacustris

Diptera
Chaoboridae (larvae of *Chaoborus sp.*)
Chironomidae (larvae)

Hirudinea
Helobdella stagnalis
Herpobdella octoculata

Mollusca
Planorbidae
Gyraulus albus

There were no consistent concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L (20 µg TI-435 50WG/L), on benthic macroinvertebrates (MASS units) taxa abundance, diversity, evenness, similarity, principle response curves or population densities (p. 132; Figures 36-37, pp. 133-135; p. 136; Figures 38-39, pp. 137-138; p. 139; Figure 40, p. 139; p. 176; Figures 56-59, pp. 177-184; Attachment II, Tables 125-127, p. B73; Table 131, p. B75).

Summary of effects of clothianidin (TI-435) on benthic macroinvertebrates recovered in MASS[1] units

Parameter/Taxon	NOEC[2] (µg a.i./L)	Complete Recovery (days posttreatment)
Number of taxa	10	No toxic effect.
Diversity	10	No toxic effect.
Evenness	10	No toxic effect.
Steinhaus' Similarity	10	No toxic effect.
Stander's Similarity	10	No toxic effect.
PRC[3] analysis	10	No toxic effect.
Chironomidae larvae	10	No toxic effect.
Gyraulus albus (water snail)	10	No toxic effect.
Helobdella stagnalis (leech)	10	No toxic effect.
Tubificidae (worms)	10	No toxic effect.

1 Macroinvertebrate Artificial Substrate Sampler.
2 No Observed Effect Concentration; based on Williams tests (one-sided smaller, $\alpha = 0.05$; Attachment II, Table 131, p. B75).
3 Principle Response Curve.
Data were obtained from p. 22; Attachment II, Table 131, p. B75 of the study report.

4. Benthic macroinvertebrates recovered in sediment: The following benthic organisms (5 taxa) were identified (Table 14, p. 142):

Annelida
Oligochaeta
Tubificidae

Diptera
Chironomidae (larvae)

Hirudinea
Helobdella stagnalis

Mollusca
Lymnaeidae
Radix ovata
Planorbidae
Gyraulus albus

There were no consistent concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L (20 µg TI-435 50WG/L), on sediment dwelling benthic macroinvertebrates taxa abundance or similarity; however, diversity and evenness showed notable decreases at both the 3.1 and 10 µg a.i./L treatment rates (6.3 and 20 µg TI-435 50WG/L, respectively) at 7 and 14 days, with evenness at 10 µg a.i./L decreasing again at 42 and 98 days (pp. 140, 144; Figures 41-

43, pp. 141-144; Attachment II, Tables 132-134, p. B76). Based on Williams tests, diversity was significantly reduced at 7 days and evenness at 98 days (both NOEC 3.1 µg a.i./L, 6.3 µg TI-435 50WG/L); however, the decrease in evenness at the highest treatment rate at 98 days was not considered a toxic effect (p. 140; Attachment II, Table 137, p. B77). Concentration-effect analyses (7-14 day posttreatment interval) yielded statistically significant EC20 and EC50 values of 2.0 µg a.i./L (3.9 µg TI-435 50WG/L) and 9.2 µg a.i./L (18.4 µg TI-435 50WG/L), respectively, for diversity and 3.3 µg a.i./L (6.5 µg TI-435 50WG/L) and 15.3 µg a.i./L (30.6 µg TI-435 50WG/L), respectively, for evenness (p. 140; Figure 44, p. 146; Table 16, p. 147). The decreases in diversity and evenness were most likely due to toxic effects of clothianidin on chironomid larvae at the two highest treatment rates; NOEC of 1.0 µg a.i/L (2.0 µg TI-435 50WG/L) at 7 days posttreatment and 3.1 µg a.i/L (6.3 µg TI-435 50WG/L) at 14 days based on Williams tests (pp. 140, 187; Figure 63, pp. 192-193; Attachment II, Table 137, p. B77). There were no consistent concentration-dependent toxic effects on population densities of the other recovered benthic macroinvertebrate taxa (tubificid worms, water snails, leeches; p. 185; Figure 60, pp. 186-187; Figures 61-62, pp. 188-191; Attachment II, Table 137, p. B77). Principle response curve analysis was not conducted due to the low number of taxa (≤ 5) recovered (p. 147).

Effective concentration values (EC20, EC50) for clothianidin (as TI-435 50WG) for benthic macroinvertebrates recovered in sediment[1]

Parameter	Interval[2]	a	b	R^2	Significance	EC20		EC50	
						(µg TI-435 50WG/L)	(µg a.i./L)	(µg TI-435 50WG/L)	(µg a.i./L)
Taxa abundance	Pre			n.d.[3]					
	Treat	10813737	0.144	0.115					
	Post1			n.d.					
	Post2			n.d.					
Diversity	Pre			n.d.					
	Treat	18.404	0.894	0.879	$p \leq 0.05$	3.9	2.0	18.4	9.2
	Post1			n.d.					
	Post2			n.d.					
Evenness	Pre			n.d.					
	Treat	30.582	0.897	0.873	$p \leq 0.05$	6.5	3.3	30.6	15.3
	Post1	118.117	0.903	0.072		(25.4)[4]	(12.7)	(118.1)	(59.1)
	Post2			n.d.					

1 Determined by study author using two-parameter nonlinear regression analysis fitted with the Downhill-simplex algorithm (pp. 56-59; Figure 44, p. 146; Table 16, p. 147 of the study report).

2 Pre = pre-treatment period: day 0,
Treat = treatment period: 7 to 14 days posttreatment,
Post1 = posttreatment period 1: 28 to 56 days posttreatment,
Post2 = posttreatment period 2: 70 to 98 days posttreatment.

3 Not determined.

4 Values in parentheses from non-significant concentration-effect relationships.

Data were obtained from Table 16, p. 147 of the study report.

Summary of effects of clothianidin (TI-435) on benthic macroinvertebrates recovered in sediment

Parameter/Taxon	NOEC[1] (µg a.i./L)	Complete Recovery (days posttreatment)
Number of taxa	10	No toxic effect.
Diversity	1	28
Evenness	1	28-56
Steinhaus' Similarity	10	No toxic effect.
Stander's Similarity	10	No toxic effect.
Chironomidae larvae	1	28
Gyraulus albus (water snail)	10	No toxic effect.
Helobdella stagnalis (leech)	10	No toxic effect.
Tubificidae (worms)	10	No toxic effect.

1 No Observed Effect Concentration; based on Williams tests (one-sided smaller, $\alpha = 0.05$; Attachment II, Table 137, p. B77).

Data were obtained from p. 22; Attachment II, Table 137, p. B77 of the study report.

5. Emergent insects: The following emergent insects (16 taxa) were identified (p. 119; Table 9, p. 120):

Diptera
- Chaoboridae
  - *Chaoborus sp.* (probably *Chaoborus crystallinus*)
- Chironomidae
  - Chironominae
    - *Chironominae* (females)
    - *Chironomus sp.*
    - *Dicrotendipes sp.*
    - *Procladius sp.*
  - Orthocladiinae
    - *Orthocladiinae* (females)
    - *Paraphaenocladius sp.*
    - *Psectrocladius sp.*
  - Tanypodinae
    - *Cricotopus sp.*
    - *Psectrotanypes sp.*
    - *Tanypodinae* (females)
- Culicidae
  - *Culex sp.*
- Ephydridae
  - *Clanoneurum sp.*
- Nematocera
  - *Nematocera* (females)

Ephemeroptera
Caenis horaria
Cloeon dipterum

There were no consistent concentration-dependent toxic effects attributable to clothianidin up to 1 µg a.i./L (2 µg TI-435 50WG/L) on emergent insect taxa abundance, diversity, evenness or similarity; however, at both 3.1 and 10 µg a.i/L (6.3 and 20 µg TI-435 50WG/L, respectively), all population parameters showed transient toxic effects, with recovery to control levels occurring by 63-70 days posttreatment (p. 126).

Taxa abundance at both the 3.1 and 10 µg a.i/L rates notably decreased at 7-21 days posttreatment, with recovery to control levels by 28 days for the 3.1 µg a.i./L rate and by 63-70 days for the 10 µg a.i./L rate (pp. 119-120; Figure 31, pp. 121-122). Initial calculated EC20 and EC50 values were 1.2 and 3.1 µg a.i./L, respectively, (2.3 and 6.2 µg TI-435 50WG/L, respectively; Table 11, p. 129). Based on Williams tests, mean taxa abundance were significantly lower than controls at the 10 µg a.i./L rate at 7 days posttreatment (NOEC 3.1 µg a.i./L), and at both the 3.1 and 10 µg a.i./L rates at 14 and 21 days (NOEC 1.0 µg a.i./L; p. 120; Attachment II, Table 124, p. B72).

Similarly, diversity and evenness at both the 3.1 and 10 µg a.i/L rates notably decreased at 7-21 days posttreatment, with recovery to control levels by 28 days for the 3.1 µg a.i./L rate and by 63-70 days for the 10 µg a.i./L rate (p. 122; Figure 32, p. 123). Initial calculated EC20 and EC50 values were 1.4 and 2.9 µg a.i./L, respectively, (2.7 and 5.8 µg TI-435 50WG/L, respectively) for diversity, and 3.1 and 3.8 µg a.i./L, respectively, (6.2 and 7.5 µg TI-435 50WG/L, respectively) for evenness (Table 11, p. 129). Based on Williams tests, evenness was significantly lower than controls at the 10 µg a.i./L rate at 7-21 days (NOEC 3.1 µg a.i./L), while diversity was significantly lower than controls at the 10 µg a.i./L rate at 7 days posttreatment (NOEC 3.1 µg a.i./L) and at both the 3.1 and 10 µg a.i./L rates at 14 and 21 days (NOEC 1.0 µg a.i./L; Attachment II, Table 124, p. B72). Diversity and evenness were also significantly lower than controls at the 10 µg a.i./L rate at 56 days (NOEC 3.1 µg a.i./L; Attachment II, Table 124, p. B72).

Steinhaus's similarity was notably decreased at 14-21 days posttreatment for the 3.1 µg a.i./L rate, while decreased at 7-28 days posttreatment for the 10 µg a.i./L rate, with recovery to control levels by 35 days for both rates (p. 124; Figure 33, p. 126). Stander's similarity was notably decreased only at the 10 µg a.i./L rate at 7-21 days posttreatment, with recovery to control levels by 28-35 days (p. 124; Figure 33, p. 126). For Steinhaus' similarity, initial calculated EC20 and EC50 values were 2.2 and 3.0 µg a.i./L, respectively, (4.3 and 5.9 µg TI-435 50WG/L, respectively); EC20 and EC50 values for Stander's similarity were determined (see below), but were not statistically significant for the initial posttreatment (7-21 day) interval (Table 11, p. 129).

Principle response curve analyses found decreases at all test concentrations at 7-21 days posttreatment; however, decreases were only statistically significant at both the 3.1 and 10 µg a.i./L rates at 14-21 days, recovery to control levels by 63 days (Figure 35, p. 130; p. 131).

As with other parameters, there were no consistent concentration-dependent toxic effects attributable to clothianidin up to 1 µg a.i./L (2 µg TI-435 50WG/L) on total emergent insect population densities; however, at 3.1 and 10 µg a.i/L (6.3 and 20 µg TI-435 50WG/L, respectively), total emergent population densities were significantly reduced at the 10 µg a.i./L rate at 7 days and at both the 3.1 and 10 µg a.i./L rates at 14 and 21 days, with recovery to control levels by 42 days posttreatment (p. 168; Figure 54, pp. 169-170; Attachment II, Table 124, p. B72). Initial (7-21 days) calculated EC20 and EC50 values were 1.0 and 1.7 µg a.i./L, respectively, (2.0 and 3.4 µg TI-435 50WG/L, respectively), but were not statistically significant (Table 17, p. 175).

Considering individual emergent insect taxa, the insect populations were dominated by chironomid taxa (10 of 16 total taxa; pp. 119, 148). Chironomid populations were significantly reduced at the 10 µg a.i./L rate at 14 days and at both the 3.1 and 10 µg a.i./L rates at 21 days, with recovery to control levels by 70 days (p. 149; Figures 45-46, pp. 151-154; Attachment II, Table 124, p. B72). For *Chironominae* (females), initial (7-21 days) calculated EC20 and EC50 values were 0.9 and 1.2 µg a.i./L, respectively, (1.7 and 2.3 µg TI-435 50WG/L, respectively); EC20 and EC50 values for *Chironomus sp.* were determined (see below), but were not statistically significant for the initial posttreatment (7-21 day) interval (Table 17, p. 175).

Cricotopus sp. populations were significantly reduced at the 10 µg a.i./L rate at 7 days and at both the 3.1 and 10 µg a.i./L rates at 14-28 days, with recovery to control levels by 21-28 days for the 3.1 µg a.i./L rate and by 35-42 days for the 10 µg a.i./L rate (p. 150; Figure 47, pp. 155-156; Attachment II, Table 124, p. B72). Initial (7-21 days) calculated EC20 and EC50 values were 0.6 and 1.5 µg a.i./L, respectively, (1.2 and 3.0 µg TI-435 50WG/L, respectively; Table 17, p. 175).

Orthocladiinae (females) populations were significantly reduced at the 10 µg a.i./L rate at 7 and 21 days and at both the 3.1 and 10 µg a.i./L rates at 14 days, with recovery to control levels by 21-28 days for the 3.1 µg a.i./L rate and by 28-35 days for the 10 µg a.i./L rate (p. 150; Figure 48, pp. 157-158; Attachment II, Table 124, p. B72). Initial (7-21 days) calculated EC20 and EC50 values were not statistically significant (see below).

Psectrocladius sp. populations were significantly reduced at both the 3.1 and 10 µg a.i./L rates at 14-21 days, with recovery to control levels by 42 days for the 3.1 µg a.i./L rate and by 77 days for the 10 µg a.i./L rate (p. 150; Figure 49, pp. 159-160; Attachment II, Table 124, p. B72). Initial (7-21 days) calculated EC20 and EC50 values were 0.2 and 0.5 µg a.i./L, respectively, (0.3 and 0.9 µg TI-435 50WG/L, respectively), but considered tentative due to low population density and high variability (Table 17, p. 175).

Results regarding the remaining emergent insect taxa were tentative due to low population densities throughout the study period (pp. 162, 168).

Effective concentration values (EC20, EC50) for clothianidin (as TI-435 50WG) for emergent insects[1]

Parameter	Interval[2]	a	b	R^2	Significance	EC20		EC50	
						(µg TI-435 50WG/L)	(µg a.i./L)	(µg TI-435 50WG/L)	(µg a.i./L)
Taxa abundance	Pre			n.d.[3]					
	Treat	6.219	1.377	0.921	$p \leq 0.05$	2.3	1.2	6.2	3.1
	Post1	20.611	14.842	1.000	$p \leq 0.05$	18.8	9.4	20.6	10.3
	Post2			n.d.					
Shannon-Weaver diversity	Pre			n.d.					
	Treat	5.759	1.786	0.949	$p \leq 0.05$	2.7	1.4	5.8	2.9
	Post1	20.669	14.579	1.000	$p \leq 0.05$	18.8	9.4	20.7	10.4
	Post2			n.d.					
Evenness	Pre			n.d.					
	Treat	7.531	7.017	0.999	$p \leq 0.05$	6.2	3.1	7.5	3.8
	Post1	25.362	4.411	0.912	$p \leq 0.05$	18.5	9.3	25.4	12.7
	Post2			n.d.					
Steinhaus' similarity	Pre			n.d.					
	Treat	5.867	4.473	1.000	$p \leq 0.05$	4.3	2.2	5.9	3.0
	Post1	22.817	12.647	1.000	$p \leq 0.05$	20.4	10.2	22.8	11.4
	Post2			n.d.					
Stander's similarity	Pre			n.d.					
	Treat	18.397	1.564	0.849		(7.6)[4]	(3.8)	(18.4)	(9.2)
	Post1	20.619	18.447	1.000	$p \leq 0.05$	19.1	9.6	20.6	10.3
	Post2			n.d.					
Chironomus sp.	Pre			n.d.					
	Treat	4.893	10.389	0.494		(4.3)	(2.2)	(4.9)	(2.5)
	Post1	5.058	14.973	0.900	$p \leq 0.05$	4.6	2.3	5.1	2.6
	Post2			n.d.					
Chironominae (females)	Pre			n.d.					
	Treat	2.312	4.418	0.971	$p \leq 0.05$	1.7	0.9	2.3	1.2
	Post1	3.153	7.645	0.967	$p \leq 0.05$	2.6	1.3	3.2	1.6
	Post2			n.d.					
Cricotopus sp.	Pre			n.d.					
	Treat	3.022	1.445	0.877	$p \leq 0.05$	1.2	0.6	3.0	1.5
	Post1	5.797	0.391	0.626		(0.2)	(0.1)	(5.8)	(2.9)
	Post2	218895.976	0.233	0.067					
Orthocladiinae (females)	Pre			n.d.					
	Treat	2.955	0.926	0.655		(0.7)	(0.4)	(3.0)	(1.5)
	Post1	5.323	0.000	-4.838					
	Post2			n.d.					
Psectrocladius sp.	Pre	0.000	0.009	0.000					
	Treat	0.918	1.126	0.959	$p \leq 0.05$	0.3[5]	0.2[5]	0.9[5]	0.5[5]
	Post1			n.d.					
	Post2			n.d.					
Chaoborus sp.	Pre			n.d.					
	Treat	3.699	1.791	0.988	$p \leq 0.05$	1.7[5]	0.9[5]	3.7[5]	1.9[5]

Effective concentration values (EC20, EC50) for clothianidin (as TI-435 50WG) for emergent insects[1]

Parameter	Interval[2]	a	b	R^2	Significance	EC20		EC50	
						(µg TI-435 50WG/L)	(µg a.i./L)	(µg TI-435 50WG/L)	(µg a.i./L)
	Post1	1.844	0.000	-0.220					
	Post2			n.d.					
Psectrotanypes sp.	Pre			n.d.					
	Treat			n.d.					
	Post1			n.d.					
	Post2			n.d.					
Tanypodinae (females)	Pre			n.d.					
	Treat			n.d.					
	Post1			n.d.					
	Post2			n.d.					
Clanoneurum sp.	Pre			n.d.					
	Treat			n.d.					
	Post1			n.d.					
	Post2	6.181	0.000	-21.911					
Total emergence	Pre			n.d.					
	Treat	3.396	2.577	0.825		(2.0)	(1.0)	(3.4)	(1.7)
	Post1	15.405	0.819	0.877	$p \leq 0.05$	2.8	1.4	15.4	7.7
	Post2			n.d.					

1 Determined by study author using two-parameter nonlinear regression analysis fitted with the Downhill-simplex algorithm (pp. 56-59; Figure 34, pp. 127-128; Table 11, p. 129; Table 17, p. 175 of the study report).

2 Pre = pre-treatment period: -14 to 0 days,
Treat = treatment period: 7 to 21 days posttreatment,
Post1 = posttreatment period 1: 28 to 63 days posttreatment,
Post2 = posttreatment period 2: 70 to 98 days posttreatment.

3 Not determined.

4 Values in parentheses from non-significant concentration-effect relationships.

5 Calculated result tentative due to very low density and high variability of the species (Table 17, p. 175).

Data were obtained from Table 11, p. 129; Table 17, p. 175 of the study report.

Summary of effects of clothianidin (TI-435) on emergent insects

Parameter/Taxon	NOEC[1] (µg a.i./L)	Complete Recovery (days posttreatment)
Number of taxa	1	63
Diversity	1	63-70
Evenness	1	63-70
Steinhaus' Similarity	1	35
Stander's Similarity	3.1	28-35
PRC[2] analysis	1	63
Chaoboridae	1-10	56
Chironominae	1	70
Ephydridae	10	No toxic effect.
Orthocladiinae	1	28-77
Tanypodinae	1-10	35-42
Total emergence	1	42

1 No Observed Effect Concentration; based on Williams tests (one-sided smaller, $\alpha = 0.05$; Attachment II, Table 124, p. B72).

2 Principle Response Curve.

Data were obtained from p. 21; Attachment II, Table 124, p. B72 of the study report.

6. Macrophytes and periphyton: No significant development of macrophytes occurred on the sediment of either the treated or control ponds throughout the study period (p. 266). However, the floating macrophyte *Lemna sp.* and growth of filamentous periphyton on the pond walls occurred in all ponds. To prevent the *Lemna sp.* and periphyton from significantly influencing water chemistry, nutrient concentrations and light conditions (shading), the plants were periodically removed. As clothianidin was not expected to have any toxic effect, only total biomass of the plants was monitored. There were no observable concentration-dependent toxic effects attributable to clothianidin, up to 10 µg a.i./L (20 µg TI-435 50WG/L), on total biomass of *Lemna sp.* and filamentous periphyton (p. 226; DER Attachment 2).

TRANSFORMATION PATHWAY: Neither water, sediment nor biota were analyzed for transformation products of clothianidin.

Table 14: Chemical names and CAS numbers for the transformation products of clothianidin (TI-435).[1]

Applicant Code Name	CAS Number	Chemical Name	Chemical Formula	MW (g/mol)	Smiles String
No transformation products were identified.					

1 Water and sediment were analyzed only for parent clothianidin, and biota were not subjected to residue analyses.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: Method validation for clothianidin in water. Recoveries from tap water (used for application solutions, HPLC/UV analysis) fortified with clothianidin at 0.350 and 46.6 mg a.i./L (0.709 and 94.5 mg TI-435 50WG/L, respectively) averaged (n = 2) 102 ± 1% (range 100-103%) of the applied (DER Attachment 2). For pond water samples analyzed via HPLC/UV, overall recovery from pond water fortified with clothianidin at 0.105-10.48 µg a.i./L (0.213-21.26 µg TI-435 50WG/L) averaged (n = 8) 95 ± 6% (range 84-103%) of the applied (DER Attachment 2). For pond water samples analyzed via LC/MS/MS, overall recovery from pond water fortified with clothianidin at 5.24 µg a.i./L (10.63 µg TI-435 50WG/L) averaged (n = 2) 88 ± 0% (range 88-89%) of the applied; however, recoveries from pond water fortified at 0.0262-0.262 µg a.i./L (0.0532-0.532 µg TI-435 50WG/L) only averaged (n = 14) 64 ± 5% (range 56-73%) of the applied (DER Attachment 2). The lower LC/MS/MS recoveries were not due to losses of clothianidin during the SEP extraction/concentration procedures, but believed due to concentration (samples concentrated 50x or 100x) of existing matrix compounds that possibly influenced the ionization behavior of clothianidin (Attachment I, pp. A23-A24). Consequently, results from all pond water samples analyzed via LC/MS/MS for this study were corrected by the mean recovery value of 64% (Attachment I, p. A24; Table 4, pp. A37-A38). Detector responses were linear for clothianidin over ranges of 0.1388-10.4088 µg/L (r^2 = 1) for the tap water samples, 2.082-55.52 µg/L (r^2 = 0.9999) for pond water samples analyzed by HPLC/UV and 0.8328-27.76 µg/L (r^2 = 0.9955) for pond water samples analyzed by LC/MS/MS (Attachment I, Tables 1a-1c, pp. A31-A33). A chromatogram of unfortified control pond water analyzed by LC/MS/MS indicated there were no significant interfering peaks at the retention time for clothianidin; no additional chromatograms of control water samples or reagent blank samples were provided (Attachment I, Figure 17, p. A53).

Method validation for clothianidin in sediment. Overall recovery from sediment fortified with clothianidin at 7.71-84.9 µg a.i./kg (15.63-172.3 µg TI-435 50WG/kg) averaged (n = 16) 104 ± 9% (range 91-123%) of the applied (DER Attachment 2). Detector responses were linear (r^2 = 0.998) for clothianidin over a range of 2.034-20.34 µg/L (Attachment I, Table 6, p. A40). Chromatograms of unfortified control sediment and reagent blank samples were not provided.

III. STUDY DEFICIENCIES: The following deviations from good scientific practices and/or the objectives of OPPTS guidelines were noted:

- Application rates were not confirmed for the ponds treated at the 0.10 and 0.31 µg a.i./L test rates. Following spray application, water samples were not taken until 2 days posttreatment, at which time clothianidin comprised only 59 ± 5% and 79 ± 2% of the applied in the 0.10 and 0.31 µg a.i./L treated ponds, respectively. Analysis of the formulated test solutions, taken just prior to application, indicated no degradation of clothianidin; however, no verification procedures were performed during spray application of the formulated solution to the test ponds.

- This pond mesocosm study was submitted to satisfy the requirements for USEPA guideline OPPTS 850.1950 Field Testing for Aquatic Organisms. However, significant differences between this study and the guideline requirements were a) water concentration analyses were not performed at study initiation, b) size of the test mesocosms, c) insufficient sediment depth, d) omission of finfish species, e) number of pond replicates for test material treatment levels and f) incomplete residue analyses. OPPTS guidelines specify:
  a) The concentration of the test material in the water should be determined at the start of the study. In this study, water was not sampled until 2 days posttreatment and application rates at the two lowest test concentrations (0.10 and 0.31 µg a.i./L) were not confirmed.
  b) A minimum mesocosm volume of 300 m^3. In this study, mesocosm volumes were only *ca*. 3.4-4.2 m^3 (based on water surface areas of *ca*. 3.1 m^2 for ten of the ponds and *ca*. 3.8 m^2 for three of the ponds, and a water depth of *ca*. 1.1 m for all ponds; p. 27).
  c) Sediment depth should be a minimum of 15 cm. In this study, sediment depth was *ca*. 10 cm.
  d) Mesocosm dimensions must be sufficient to accommodate a viable finfish population. In this study, the pond systems, by design, were too small to accommodate finfish (p. 38).
  e) A minimum of three replicates per treatment level, *i.e.* a three-replicate by four-treatment level design. In this study, only two replicates per treatment level were conducted for clothianidin application; three replicates for the untreated control ponds were utilized.
  f) Residues of the test material and major degradates/metabolites are to be analyzed at appropriate intervals in the water, sediments and biota. In this study, water and sediments were only analyzed for parent clothianidin, and biota were not subjected to any residue analyses.

  Other differences from OPPTS guidelines that may or may not be of significance include:

  g) Test conditions should resemble the conditions likely to be encountered under actual use. Clothianidin was described as an insecticide (p. 226); however, the purpose for applying the test substance to the pond systems was not clearly stated; such as, for the purpose of simulating spray drift during a field application.
  h) A minimum of four treatment levels consisting of an untreated control, an X treatment level representing expected exposure, an X+ treatment level representing an upper bound exposure level (10x of expected exposure recommended), and an X- treatment level representing a lower bound exposure level (1/10 of expected exposure recommended). In this study, five application rates (0.1-10 µg a.i./L) were employed; however, the "expected exposure level" was not specified.

IV. REVIEWER'S COMMENTS

1. All mean results and standard deviations presented in this review were determined by the primary reviewer using Microsoft Excel 2002 (10.2614.2625) 2002 (10.2614.2625) and/or 2007 (12.0.6024.5000) MSO (12.0.6017.5000) software (DER Attachment 2). Standard deviations were determined using the "biased" or "n" method which determines the standard deviation of the entire sample population.

   Regarding clothianidin residues in water and sediment presented in the study report, the results were calculated based on the amount of TI-435 50WG formulated product added to the test systems and not on the actual amount of clothianidin active ingredient added (Attachment I, p. A28; Table 4, pp. A36-A38). Consequently, for all clothianidin residue results presented in this DER, the primary reviewer recalculated the provided data to reflect residue levels of the active ingredient (DER Attachment 2).

   Additionally, there was no consistent use of significant digits and/or decimal place in the reported results. Therefore, the primary reviewer used all results as reported, with the exception of the results for emergent insects which were reported to the hundredths (0.00) decimal place even though whole individuals were tallied (Attachment II, Table 26-42, pp. B20-B28; DER Attachment 2).

   Organism summations reported by the study author (Attachment II, Table 1, p. B7; Table 7, p. B10; Table 11, p. B12; Table 26, p. B20; Table 57, p. B39; Table 75, p. B48; Table 78, p. B49; Table 83, p. B52) were verified by the primary reviewer and, with the exception noted below, there was good agreement [±0.5 for phytoplankton, ± 0.02 for zooplankton, and ± 0.0 for emergent insects at 0-56 days posttreatment (see below); summations of benthic macroinvertebrates were not provided] between the study author's reported values and those determined by the primary reviewer (DER Attachment 2).

   - Table 26 (Attachment II, p. B20): the total emergent insect results reported for 63-98 days posttreatment are incorrect and appear to be due to data entry error; the 63-98 day results are the same as those reported in Table 28 (Attachment II, p. B21).

   - Data provided in Tables 88, 90 and 92 are redundant with Tables 89, 91 and 93 respectively (Attachment II, pp. B54-B57).

V. REFERENCES

1. U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines, No. 850.1950, Field Testing for Aquatic Organisms. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-96-135.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.

Attachment 1: Structure of Parent Compound Used and Identified

Clothianidin [C-1015, C-908, TI435, K-1142, TI-435, TI-435 50 WDG, TI-435 50WDG]

IUPAC Name: (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine. (E)-N-(2-chloro-1,3-thiazol-5-yl)methyl]-N-[oxido(oxo)hydrazono]methanediamine. Chloro-1,3-thiazol-5-yl)methyl]-N-{(E)-(methylamino)[oxido(oxo)hydrazono]methyl}amine.

CAS Name: [C(E)]-N-[(2-chloro-5-thiazolyl)methyl]-N′-methyl-N″-nitroguanidine.

CAS Number: 210880-92-5 (formerly 205510-53-8).

SMILES String: CNC(=N[N+](=O)O)NCc1cnc(Cl)s1 (Online SMILES Translator and Structure File Generator at http://cactus.nci.nih.gov/services/translate/). [O-][N+](=O)N=C(NCc1cnc(s1)Cl)NC.

Empirical formula: $C_6H_8ClN_5O_2S$ **Molecular formula:** $C_6H_8ClN_5O_2S$